Answers to Questions

Chapter 1

1.1 Basic Themes

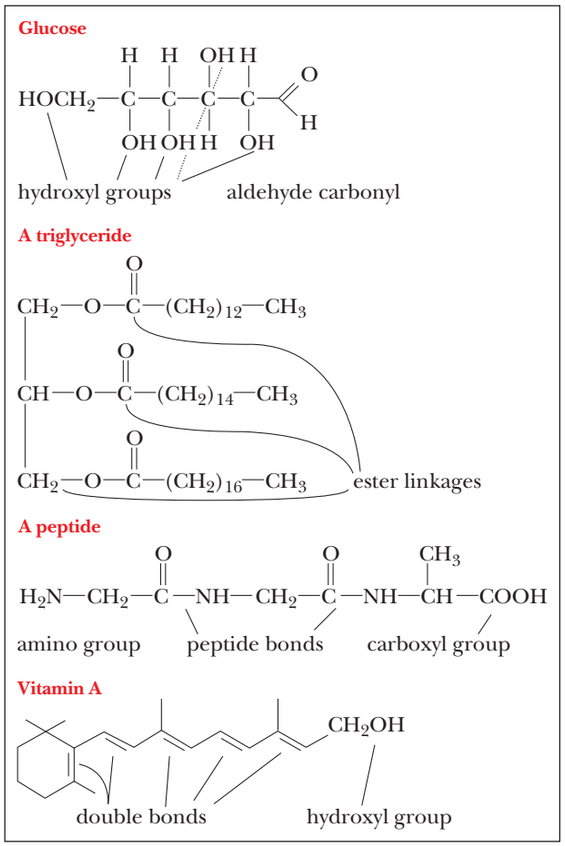
1. A polymer is a very large molecule formed by linking smaller units (monomers) together. A protein is a polymer formed by linking amino acids together. A nucleic acid is a polymer formed by linking nucleotides together. Catalysis is the process that increases the rate of chemical reactions compared with the rate of the uncatalyzed reaction. Biological catalysts are proteins in almost all cases; the only exceptions are a few types of RNA, which can catalyze some of the reactions of their own metabolism. The genetic code is the means by which the information for the structure and function of all living things is passed from one generation to the next. The sequence of purines and pyrimidines in DNA carries the genetic code. (RNA is the coding material in some viruses.)

1.2 Chemical Foundations of Biochemistry

2. The correct match of functional groups and the compounds containing those functional groups is given in the following list.

Amino group	$CH_3CH_2NH_2$
Carbonyl group (ketone)	CH_3COCH_3
Hydroxyl group	CH_3OH
Carbonyl group	CH_3COOH
Carbonyl group (aldehyde)	CH_3CH_2CHO
Thiol group	CH_3SH
Ester linkage	$CH_3COOCH_2CH_3$
Double bond	$CH_3CH{=}CHCH_3$
Amide linkage	$CH_3CON(CH_3)_2$
Ether	$CH_3CH_2OCH_2CH_3$

3. The functional groups in the compounds follow:



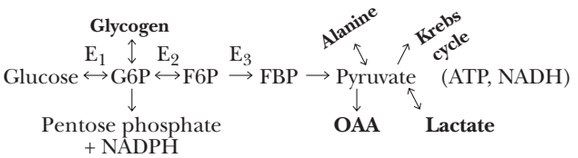
4. Before 1828, the concept of vitalism held that organic compounds could be made only by living systems and were beyond the realm of laboratory investigations. Wöhler's synthesis showed that organic compounds, like inorganic ones, did not require a vitalistic explanation, but that, rather, they obeyed the laws of chemistry and physics and thus were subject to laboratory investigation. Subsequently, the concept was extended to the much more complex, but still testable, discipline of biochemistry.
5. Urea, like all organic compounds, has the same molecular structure, whether it is produced by a living organism or not.
6.

Item	Organic	Biochemical
Solvent	Varies (smelly)	Water (usually)
Concentrations	High	Low (mM, μM, nM)
Use catalyst?	Usually not	Almost always (enzymes)
Speed	Min, hr, day	μsec, nsec
Temp	Varies (high)	Isothermal, ambient temp
Yield	Poor–good (90%)	High (can be 100%)
Side reactions	Often*	No
Internal control	Little	Very high**—choices
Polymers (product)	Usually not	Commonly (proteins, nucleic acids, saccharides)
Bond strength	High (covalent)	High, weak (in polymers)
Bond distances	Not critical	Critical (close fit)
Compartmented	No	Yes (esp. eukaryotes)
Emphasis	One reaction	Pathways, interconnected (control** choices)†
System	Closed or open	Open (overcome $+\Delta G$)

* Example of side reactions: Glucose → G6P *or* G1P *or* G2P.

** Control levels: enzyme, hormone, gene.

† Example of choices:



7. Five; seven if the two cyclopropane derivatives are allowed.
8. Thirteen different alcohols, 11 aldehydes/ketones, and 10 each epoxides and ethers.

1.3 The Beginnings of Biology: Origin of Life

9. It is generally believed that carbon is the likely basis for all life forms, terrestrial or extraterrestrial.
10. Eighteen residues would give 20^{18}, or 2.6×10^{23} possibilities. Thus, 19 residues would be necessary to have at least Avogadro's number (6.022×10^{23}) of possibilities.
11. The number is 4^{40}, or 1.2×10^{24}, which is twice Avogadro's number.
12. RNA is capable of both coding and catalysis.
13. Catalysis allows living organisms to carry out chemical reactions much more efficiently than without catalysts.
14. Two of the most obvious advantages are speed and specificity; they also work at constant temperature or produce little heat.
15. Coding allows for reproduction of cells.

16. With respect to coding, RNA-type polynucleotides have been produced from monomers in the absence of either a preexisting RNA to be copied or an enzyme to catalyze the process. The observation that some existing RNA molecules can catalyze their own processing suggests a role for RNA in catalysis. With this dual role, RNA may have been the original informational macromolecule in the origin of life.
17. It is unlikely that cells could have arisen as bare cytoplasm without a plasma membrane. The presence of the membrane protects cellular components from the environment and prevents them from diffusing away from each other. The molecules within a cell can react more easily if they are closer to each other.

1.4 The Biggest Biological Distinction—Prokaryotes and Eukaryotes

18. Five differences between prokaryotes and eukaryotes are as follows: (1) Prokaryotes do not have a well-defined nucleus, but eukaryotes have a nucleus marked off from the rest of the cell by a double membrane. (2) Prokaryotes have only a plasma (cell) membrane; eukaryotes have an extensive internal membrane system. (3) Eukaryotic cells contain membrane-bounded organelles, while prokaryotic cells do not. (4) Eukaryotic cells are normally larger than those of prokaryotes. (5) Prokaryotes are single celled organisms, while eukaryotes can be either single-celled or multicellular.
19. Protein synthesis takes place on ribosomes both in prokaryotes and in eukaryotes. In eukaryotes, ribosomes may be bound to the endoplasmic reticulum or found free in the cytoplasm; in prokaryotes, ribosomes are only found free in the cytoplasm.

1.5 Prokaryotic Cells

20. It is unlikely that mitochondria would be found in bacteria. These eukaryotic organelles are enclosed by a double membrane, and bacteria do not have an internal membrane system. The mitochondria found in eukaryotic cells are about the same size as most bacteria.

1.6 Eukaryotic Cells

21. See Section 1.6 for the functions of the parts of an animal cell, which are shown in Figure 1.10a.
22. See Section 1.6 for the functions of the parts of a plant cell, which are shown in Figure 1.10b.
23. In green plants photosynthesis takes place in the membrane system of chloroplasts, which are large membrane-enclosed organelles. Photosynthetic bacteria have extensions of the plasma membrane into the interior of the cell called chromatophores, which are the sites of photosynthesis.
24. Nuclei, mitochondria, and chloroplasts are all enclosed by a double membrane.
25. Nuclei, mitochondria, and chloroplasts all contain DNA. The DNA found in mitochondria and in chloroplasts differs from that found in the nucleus.
26. Mitochondria carry out a high percentage of the oxidation (energy-releasing) reactions of the cell. They are the primary sites of ATP synthesis.
27. The Golgi apparatus is involved in binding carbohydrates to proteins and in exporting substances from the cell. Lysosomes contain hydrolytic enzymes, peroxisomes contain catalase (needed for the metabolism of peroxides), and glyoxysomes contain enzymes needed by plants for the glyoxylate cycle. All of these organelles have the appearance of flattened sacs, and each is enclosed by a single membrane.

1.7 Five Kingdoms, Three Domains

28. Monera includes bacteria (e.g., *E. coli*) and cyanobacteria. Protista includes such organisms as *Euglena, Volvox, Amoeba,* and *Paramecium.* Fungi includes molds and mushrooms. Plantae includes club mosses and oak trees. Animalia includes spiders, earthworms, salmon, rattlesnakes, robins, and dogs.
29. The kingdom Monera consists of prokaryotes. Each of the other four kingdoms consists of eukaryotes.
30. The kingdom Monera is divided into the domains Eubacteria and Archaea on the basis of biochemical differences. The domain Eukarya consists of the four kingdoms of eukaryotic organisms.

1.8 Common Ground for All Cells

31. The major advantage of being eukaryotic is that of having compartments (organelles) with specialized functions (and thus division of labor). Another advantage is that cells can be much larger without surface area–to–volume considerations being critical because of compartmentalization.
32. See the discussion of the endosymbiotic theory in Section 1.8.
33. See Question 32. The division of labor in cells gives rise to greater efficiency and a larger number of individuals. This in turn allows more opportunity for evolution and speciation.

1.9 Biochemical Energetics

34. Processes that release energy are favored.

1.10 Energy and Change

35. The term *spontaneous* means energetically favored. It does not necessarily mean fast.

1.11 Spontaneity in Biochemical Reactions

36. The system consists of the nonpolar solute and water, which become more disordered when a solution is formed; ΔS_{sys} is positive but comparatively small. ΔS_{surr} is negative and comparatively large because it is a reflection of the unfavorable enthalpy change for forming the solution (ΔH_{sys}).
37. Processes (a) and (b) are spontaneous, whereas processes (c) and (d) are not. The spontaneous processes represent an increase in disorder (increase in the entropy of the Universe) and have a negative ΔG° at constant temperature and pressure, while the opposite is true of the nonspontaneous processes.
38. In all cases, there is an increase in entropy, and the final state has more possible random arrangements than the initial state.
39. Since the equation involves multiplication of ΔS by T, the value of ΔG is temperature-dependent.
40. If one considers entropy a measure of dispersion of energy, then at higher temperatures, it is logical that molecules would have more possible arrangements due to increased molecular motion.
41. Assuming the value of ΔS is positive, an increase in temperature increases the $-\Delta G$ contribution of the entropy component to the overall energy change.
42. The heat exchange, getting colder, reflects only the enthalpy or ΔH component of the total energy change. The entropy change must be high enough to offset the enthalpy component and to add up to an overall $-\Delta G$.
43. Entropy would increase. Two molecules, ADP and P_i, can be randomized in more ways than a single molecule, ATP, can.

1.12 Life and Thermodynamics

44. The lowering of entropy needed to give rise to organelles leads to higher entropy in the surroundings, thus increasing the entropy of the Universe as a whole.
45. Compartmentalization in organelles brings components of reactions into proximity with one another. The energy change of the reaction is not affected, but the availability of components allows it to proceed more readily.
46. DNA would have higher entropy with the strands separated. There are two single strands instead of one double strand, and the single strands have more conformational mobility.
47. See the answer to Question 43. It is still unlikely that cells could have arisen as bare cytoplasm, but the question of proximity of reactants is more to the point here than the energy change of a given reaction.
48. It would be unlikely that cells of the kind we know would have evolved on a gas giant. The lack of solids and liquids on which aggregates could form would make a large difference.
49. The available materials differ from those that would have been found on Earth, and conditions of temperature and pressure are very different.
50. Mars, because of conditions more like those on Earth.
51. A number of energetically favorable interactions drive the process of protein folding, ultimately increasing the entropy of the Universe.
52. Photosynthesis is endergonic, requiring light energy from the Sun. The complete aerobic oxidation of glucose is exergonic and is a source of energy for many organisms, including humans. It would be reasonable to expect the two processes to take place differently in order to provide energy for the endergonic one.

Chapter 2

2.1 Water and Polarity

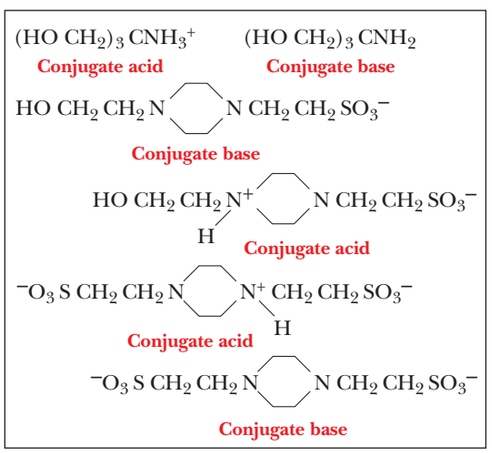
1. The unique fitness of water for forming hydrogen bonds determines the properties of many important biomolecules. Water can also act as an acid and as a base, giving it great versatility in biochemical reactions.
2. If atoms did not differ in electronegativity, there would be no polar bonds. This would drastically affect all reactions that involve functional groups containing oxygen or nitrogen—that is, most biochemical reactions.

2.2 Hydrogen Bonds

3. Proteins and nucleic acids have hydrogen bonds as an important part of their structures.
4. Replication of DNA and its transcription to RNA requires hydrogen bonding of complementary bases to the DNA template strand.
5. The C—H bond is not sufficiently polar for greatly unequal distribution of electrons at its two ends. Also, there are no unshared pairs of electrons to serve as hydrogen bond acceptors.

6. Many molecules can form hydrogen bonds. Examples might be H_2O, CH_3OH, or NH_3.
7. For a bond to be called a hydrogen bond, it must have a hydrogen covalently bonded to O, N, or F. This hydrogen then forms a hydrogen bond with another O, N, or F.
8. In a hydrogen-bonded dimer of acetic acid, the —OH portion of the carboxyl group on molecule 1 is hydrogen-bonded to the —C=O portion of the carboxyl group on molecule 2, and vice versa.
9. Glucose = 17, sorbitol = 18, ribitol = 15; each alcohol group can bond to three water molecules and the ring oxygen binds to two. The sugar alcohols bind more than the corresponding sugars.
10. Positively charged ions bind to nucleic acids as a result of electrostatic attraction to the negatively charged phosphate groups.

2.3 Acids, Bases, and pH

11. (a) $(CH_3)_3NH^+$ (conjugate acid), $(CH_3)_3N$ (conjugate base)
    (b) $^+H_3N—CH_2—COOH$ (conjugate acid), $^+H_3N—CH_2—COO^-$ (conjugate base)
    (c) $^+H_3N—CH_2—COO^-$ (conjugate acid), $H_2N—CH_2—COO^-$ (conjugate base)
    (d) $^-OOC—CH_2—COOH$ (conjugate acid), $^-OOC—CH_2—COO^-$ (conjugate base)
    (e) $^-OOC—CH_2—COOH$ (conjugate base), $HOOC—CH_2—COOH$ (conjugate acid)
12. 

13. Aspirin is electrically neutral at the pH of the stomach and can pass through the membrane more easily than in the small intestine.
14. The definition of pH is $-\log[H^+]$. By definition of the log function, a change in concentration of 10 leads to a change in pH of 1. The log of 10 is 1, the log of 100 is 2, etc.
15. Blood plasma, pH 7.4 — $[H^+] = 4.0 \times 10^{-8}$ M
    Orange juice, pH 3.5 — $[H^+] = 3.2 \times 10^{-4}$ M
    Human urine, pH 6.2 — $[H^+] = 6.3 \times 10^{-7}$ M
    Household ammonia, pH 11.5 — $[H^+] = 3.2 \times 10^{-12}$ M
    Gastric juice, pH 1.8 — $[H^+] = 1.6 \times 10^{-2}$ M
16. Saliva, pH 6.5 — $[H^+] = 3.2 \times 10^{-7}$ M
    Intracellular fluid (liver), pH 6.9 — $[H^+] = 1.6 \times 10^{-7}$ M
    Tomato juice, pH 4.3 — $[H^+] = 5.0 \times 10^{-5}$ M
    Grapefruit juice, pH 3.2 — $[H^+] = 6.3 \times 10^{-4}$ M
17. Saliva, pH 6.5 — $[OH^-] = 3.2 \times 10^{-8}$ M
    Intracellular fluid (liver), pH 6.9 — $[OH^-] = 7.9 \times 10^{-8}$ M
    Tomato juice, pH 4.3 — $[OH^-] = 2.0 \times 10^{-10}$ M
    Grapefruit juice, pH 3.2 — $[OH^-] = 1.6 \times 10^{-11}$ M

2.4 Titration Curves

18. (a) The numerical constant equal to the concentration of the products of the dissociation divided by the concentration of the undissociated acid form: $([H^+][A^-])/[HA]$.
    (b) The qualitative or quantitative description of how much acid (HA) dissociates to hydrogen ion.
    (c) The property of a molecule that has both a polar region and a nonpolar region.
    (d) The amount of acid or base that can be added to a buffer before experiencing a sharp pH change.
    (e) The point in a titration curve at which the added acid or base equals the amount of buffer originally present.
    (f) The property of a molecule that is readily soluble in water (i.e., water-loving).
    (g) The property of a molecule that is insoluble in water (i.e., water-hating).
    (h) The property of a molecule that is not soluble in water. The property of a covalent bond in which there is even sharing of electrons and no dipole moments (partial charges).
    (i) The property of a molecule that is soluble in water. The property of a covalent bond in which the electrons are not shared evenly and dipole moments (partial charges) exist.
    (j) An experiment in which acid or base is added stepwise to a solution of a compound and the pH is measured as a function of the added substance.
19. To get a titration curve most like the one in Figure 2.15, we have to titrate a compound with a pK_a as close as possible to that of $H_2PO_4^-$. According to Table 2.8, MOPS has a pK_a of 7.2, which is the closest value.
20. The titration curve for TRIS would be shifted to the right compared to that of phosphate. The crossover point would be at pH 8.3, rather than pH 7.2.

2.5 Buffers

21. The pK of the buffer should be close to the desired buffer pH, and the substance chosen should not interfere with the reaction being studied.
22. The useful pH range of a buffer is one pH unit above and below its pK_a.
23. Use the Henderson–Hasselbalch equation:

$$\text{ph} = pK_a + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$5.00 = 4.76 + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$0.24 = \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$\frac{[CH_3COO^-]}{[CH_3COOH]} = \text{inverse log of } 0.24 = \frac{1.7}{1}$$

24. Use the Henderson–Hasselbalch equation:

$$\text{pH} = pK_4 + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$4.00 = 4.76 + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$-.076 = \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$\frac{[CH_3COO^-]}{[CH_3COOH]} = \text{inverse log of } -0.76 = \frac{0.17}{1}$$

25. Use the Henderson–Hasselbalch equation:

$$\text{pH} = pK_4 + \log\left(\frac{[\text{TRIS}]}{[\text{TRIS-H}^+]}\right)$$

$$8.7 = 8.3 + \log\left(\frac{[\text{TRIS}]}{[\text{TRIS-H}^+]}\right)$$

$$0.4 = \log\left(\frac{[\text{TRIS}]}{[\text{TRIS-H}^+]}\right)$$

$$\frac{[\text{TRIS}]}{[\text{TRIS-H}^+]} = \text{inverse log of } 0.4 = \frac{2.5}{1}$$

26. Use the Henderson–Hasselbalch equation:

$$\text{pH} = pK_4 + \log\left(\frac{[\text{HEPES}]}{[\text{HEPES-H}^+]}\right)$$

$$7.9 = 7.55 + \log\left(\frac{[\text{HEPES}]}{[\text{HEPES-H}^+]}\right)$$

$$0.35 = \log\left(\frac{[\text{HEPES}]}{[\text{HEPES-H}^+]}\right)$$

$$\frac{[\text{HEPES}]}{[\text{HEPES-H}^+]} = \text{inverse log of } 0.35 = \frac{2.2}{1}$$

27. At pH 7.5, the ratio of $[HPO_4^{2-}]/[H_2PO_4^-]$ is 2/1 (pK_a of $H_2PO_4^-$ = 7.2), as calculated using the Henderson–Hasselbalch equation. K_2HPO_4 is a source of the base form, and HCl must be added to convert one-third of it to the acid form, according to the 2/1 base/acid ratio. Weigh out 8.7 g of K_2HPO_4 (0.05 mol, based on a formula weight of 174 g/mol), dissolve it in a small quality of distilled water, add 16.7 mL of 1 *M* HCl (gives 1/3 of 0.05 mol of hydrogen ion, which converts 1/3 of the 0.05 mol of HPO_4^{2-} to $H_2PO_4^-$), and dilute the resulting mixture to 1 L.

28. A 2/1 ratio of the base form to acid form is still needed, because the pH of the buffer is the same in both problems. NaH_2PO_4 is a source of the acid form, and NaOH must be added to convert two-thirds of it to the base form. Weigh out 6.0 g of NaH_2PO_4 (0.05 mol, based on a formula weight of 120 g/mol), dissolve it in a small quantity of distilled water, add 33.3 mL of 1 *M* NaOH (gives 2/3 of 0.05 mol of hydroxide ion, which converts 2/3 of the 0.05 mol of $H_2PO_4^-$ to HPO_4^{2-}), and dilute the resulting mixture to 1 L.

29. After mixing, the buffer solution (100 mL) contains 0.75 *M* lactic acid and 0.25 *M* sodium lactate. The pK_a of lactic acid is 3.86. Use the Henderson–Hasselbalch equation:

$$pH = pK_4 + \log\left(\frac{[CH_3CHOHCOO^-]}{[CH_3CHOHCOOH]}\right)$$

$$pH = 3.86 + \log\left(\frac{[CH_3CHOHCOO^-]}{[CH_3CHOHCOOH]}\right)$$

$$pH = 3.86 + \log (0.25\ M/0.75\ M)$$

$$pH = 3.86 + (-0.48)$$

$$pH = 3.38$$

30. After mixing, the buffer solution (100 mL) contains 0.25 *M* lactic acid and 0.75 *M* sodium lactate. The pK_a of lactic acid is 3.86. Use the Henderson–Hasselbalch equation:

$$pH = pK_4 + \log\left(\frac{[CH_3CHOHCOO^-]}{[CH_3CHOHCOOH]}\right)$$

$$pH = 3.86 + \log\left(\frac{[CH_3CHOHCOO^-]}{[CH_3CHOHCOOH]}\right)$$

$$pH = 3.86 + \log (0.75\ M/0.25\ M)$$

$$pH = 3.86 + (0.48)$$

$$pH = 4.34$$

31. Use the Henderson–Hasselbalch equation:

$$pH = pK_4 + \log\left(\frac{[CH_3COO^-]}{[CH_3COOH]}\right)$$

$$pH = 4.76 + \log\left(\frac{[CH_3COOH^-]}{[CH_3COOH]}\right)$$

$$pH = 4.76 + \log (0.25\ M/0.10\ M)$$

$$pH = 4.76 + 0.40$$

$$pH = 5.16$$

32. Yes, it is correct; calculate the molar amounts of the two forms and insert into the Henderson–Hasselbalch equation. (2.02 g = 0.0167 mol and 5.25 g = 0.0333 mol.)

33. The solution is a buffer because it contains equal concentrations of TRIS in the acid and free amine forms. When the two solutions are mixed, the concentrations of the resulting solution (in the absence of reaction) are 0.05 *M* HCl and 0.1 *M* TRIS because of dilution. The HCl reacts with half the TRIS present, giving 0.05 *M* TRIS (protonated form) and 0.05 *M* TRIS (free amine form).

34. Any buffer that has equal concentrations of the acid and basic forms has a pH equal to its pK_a. Therefore, the buffer from Question 33 has a pH of 8.3.

35. First calculate the moles of buffer that you have: 100 mL = 0.1 L, and 0.1 L of 0.1 *M* TRIS buffer is 0.01 mol. Since the buffer is at its pK_a, there are equal concentrations of the acid and basic form, so the amount of TRIS is 0.005 mol, and the amount of TRIS-H^+ is 0.005 mol. If you then add 3 mL of 1 *M* HCl, you will be adding 0.003 mol of H^+. This reacts as shown:

$$TRIS + H^+ \rightarrow TRIS\text{-}H^+$$

until you run out of something, which will be the H^+, since it is the limiting reagent. The new amounts can be calculated as shown:

$$TRIS\text{-}H^+ = 0.005\ mol + 0.003\ mol = 0.008\ mol$$

$$TRIS = 0.005\ mol - 0.003\ mol = 0.002\ mol$$

Now plug these values into the Henderson–Hasselbalch equation:

$$pH = 8.3 + \log ([TRIS]/[TRIS\text{-}H^+]) = 8.3 + \log (0.002/0.008)$$

$$pH = 7.70$$

36. First calculate the moles of buffer that you have (we are going to do some rounding off): 100 mL= 0.1 L, and 0.1 L of 0.1 *M* TRIS buffer is 0.01 mol. Since the buffer is at pH 7.70, we saw in Question 25 that the amount of TRIS is 0.002 mol, and the amount of TRIS-H^+ is 0.008 mol. If you then add 1 mL of 1 *M* HCl, you will be adding 0.001 mol of H^+. This reacts as shown:

$$TRIS + H^+ \rightarrow TRIS\text{-}H^+$$

until you run out of something, which will be the TRIS, since it is the limiting reagent. All the TRIS is converted to TRIS-H^+:

$$TRIS\text{-}H^+ = 0.01\ mol$$

$$TRIS = \sim 0\ mol$$

We have used up the buffer capacity of the TRIS. We now have 0.001 mol of H^+ in approximately 0.1 L of solution. This is approximately 0.01 *M* H^+.

$$pH = -\log 0.01$$

$$pH = 2.0$$

37. $[H^+] = [A^-]$ for pure acid, thus $K_a = [H^+]^2/[HA]$

$$[H^+]^2 = K_a \times [HA] \qquad -2 \log [H^+] = pK_a - \log [HA]$$

$$pH = \tfrac{1}{2}(pK_a - \log [HA])$$

38. Use the Henderson–Hasselbalch equation:

$$[Acetate\ ion]/[acetic\ acid] = 2.3/1$$

39. A substance with a pK_a of 3.9 has a buffer range of 2.9 to 4.9. It does not buffer effectively at pH 7.5.

40. Use the Henderson–Hasselbalch equation. The ratio of $[A^-]/[HA]$ would be 3981 to 1.

41. In all cases, the suitable buffer range covers a pH range of pK_a +/– 1 pH units.
    (a) Lactic acid (pK_a = 3.86) and its sodium salt: pH 2.86–4.86.
    (b) Acetic acid (pK_a = 4.76) and its sodium salt: pH 3.76–5.76.
    (c) TRIS (see Table 3.4, pK_a = 8.3) in its protonated form and its free amine form: pH 7.3–9.3.
    (d) HEPES (see Table 3.4, pK_a = 7.55) in its zwitterionic form and its anionic form: pH 6.55–8.55.

42. Several of the buffers would be suitable, namely TES, HEPES, MOPS, and PIPES; but the best buffer would be MOPS, because its pK_a of 7.2 is closest to the desired pH of 7.3.

43. Buffer concentrations are typically reported to be the sum of the two ionic forms.

44. At the equivalance point of the titration, a small amount of acetic acid remains because of the equilibrium $CH_2COOH \rightarrow H^+ + CH_2COO^-$. There is a small, but nonzero, amount of acetic acid left.

45. Buffering capacity is based on the amounts of the acid and base forms present in the buffer solution. A solution with a high buffering capacity can react with a large amount of added acid or base without drastic changes in pH. A solution with a low buffering capacity can react with only comparatively small amounts of acid or base before showing changes in pH. The more concentrated the buffer, the higher is its buffering capacity. Buffer (a) has one-tenth the buffering capacity of buffer (b), which in turn has one-tenth the buffering capacity of buffer (c). All three buffers have the same pH, because they all have the same relative amounts of the acid and base form.

46. It would be more effective to start with the HEPES base. You want a buffer at a pH above the pK_a, which means that the base form predominates when you have finished preparing it. It is easier to convert some of the base form to the acid form than most of the acid form to the base form.
47. In a buffer with the pH above the pK_a, the base form predominates. This would be useful as a buffer for a reaction that produces H^+ because plenty of the base form will be available to react with the hydrogen ion produced.
48. Zwitterions tend not to interfere with biochemical reactions.
49. It is useful to have a buffer that maintains a stable pH even if assay conditions change. Dilution is one such possible change.
50. It is useful to have a buffer that maintains a stable pH even if assay conditions change. Temperature variation is one such possible change.
51. The only zwitterion is $^+H_3N—CH_2—COO^-$.
52. Hypoventilation decreases the pH of blood.

Chapter 3

3.1 Amino Acids Exist in a Three-Dimensional World

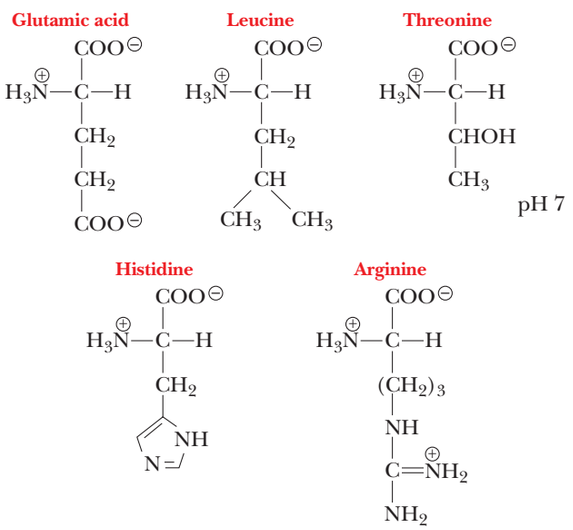
1. D- and L-amino acids have different stereochemistry around the α-carbon. Peptides that contain D-amino acids are found in bacterial cell walls and in some antibiotics.

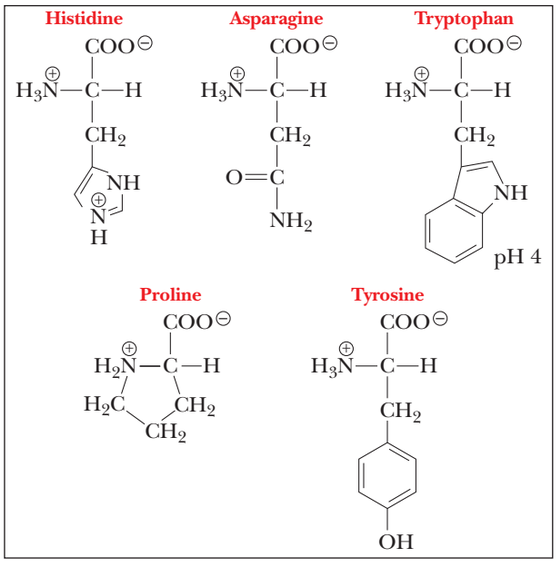
3.2 Individual Amino Acids: Their Structures and Properties

2. Proline is technically not an amino acid. Glycine contains no chiral carbon atoms.
3. Listed here are amino acids in which the R group contains the following: a hydroxyl group (serine, threonine, or tyrosine); a sulfur atom (cysteine or methionine); a second chiral carbon atom (isoleucine or threonine); an amino group (lysine); an amide group (asparagine or glutamine); an acid group (aspartate or glutamate); an aromatic ring (phenylalanine, tyrosine, or tryptophan); a branched side chain (leucine or valine).
4. In the peptide Val—Met—Ser—Ile—Phe—Arg—Cys—Tyr—Leu, the polar amino acids are Ser, Arg, Cys, and Tyr; the aromatic amino acids are Phe and Tyr; and the sulfur-containing amino acids are Met and Cys.
5. In the peptide Glu—Thr—Val—Asp—Ile—Ser—Ala, the nonpolar amino acids are Val, Ile, and Ala; the acidic amino acids are Glu and Asp.
6. Amino acids other than the usual 20 are produced by modification of one of the common amino acids. See Figure 3.5 for the structures of some modified amino acids. Hydroxyproline and hydroxylysine are found in collagen; thyroxine is found in thyroglobulin.

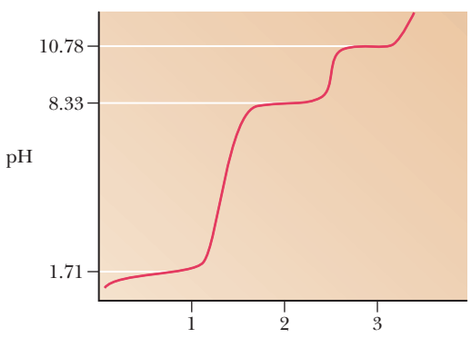
3.3 Amino Acids Can Act as Both Acids and Bases

7. The ionized forms of each of the following amino acids at pH 7—glutamic acid, leucine, threonine, histidine, and arginine—are as follows:

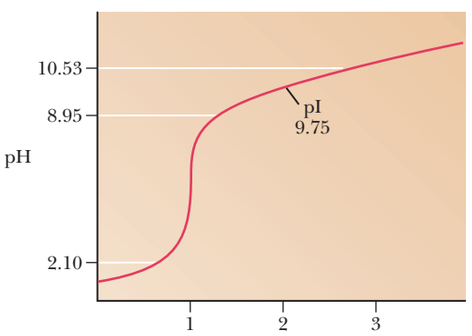




9. Histidine: imidazole is deprotonated, α-amino group is predominantly deprotonated. Asparagine: α-amino group is deprotonated. Tryptophan: α-amino group is predominantly deprotonated. Proline: α-amino group is partially deprotonated. Tyrosine: α-amino group is predominantly deprotonated, phenolic hydroxyl is approximately a 50–50 mixture of protonated and deprotonated forms.
10. Glutamic acid, 3.25; serine, 5.7; histidine, 7.58; lysine, 9.75; tyrosine, 5.65; arginine, 10.75.
11. Cysteine has no net charge at pH 5.02 = (1.71 + 8.33)/2 (see titration curve below).

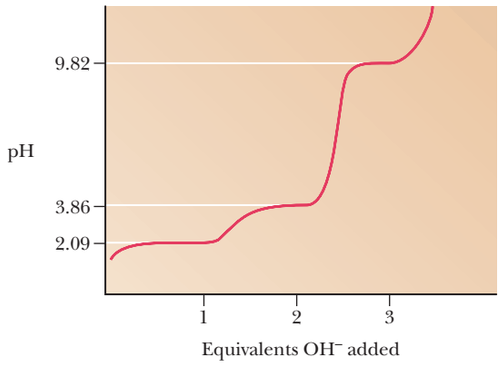


12.



13. In all cases, the yield is 0.95^n. For 10 residues, that means 60% yield; for 50 residues, 8%; and for 100 residues, 0.6%. These are not satisfactory yields. Enzyme specificity gets around the problem.

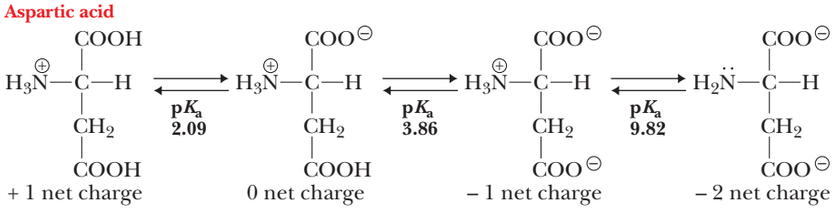
14. The conjugate acid–base pair acts as a buffer in the pH range 1.09–3.09.



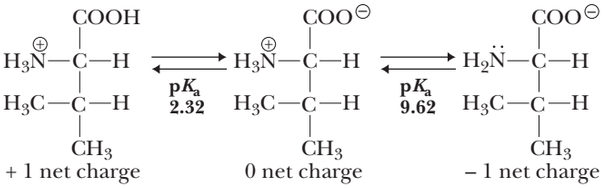
15. They have a net charge at pH extremes, and the molecules tend to repel each other. When the molecular charge is zero, the amino acids can aggregate more easily.

16. The ionic dissociation reactions of the amino acids aspartic acid, valine, histidine, serine, and lysine are as follows:

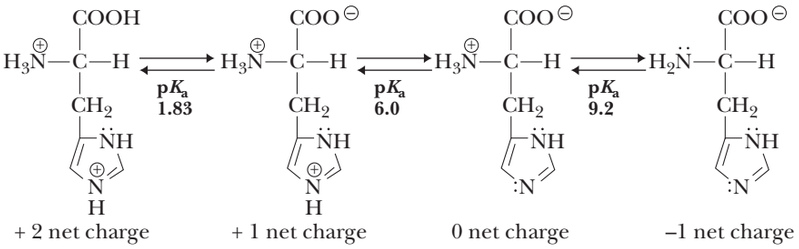
Aspartic acid



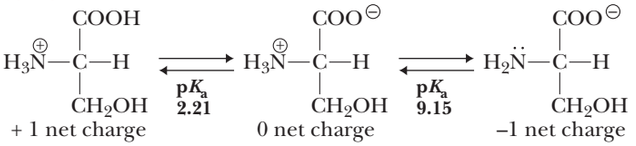
Valine



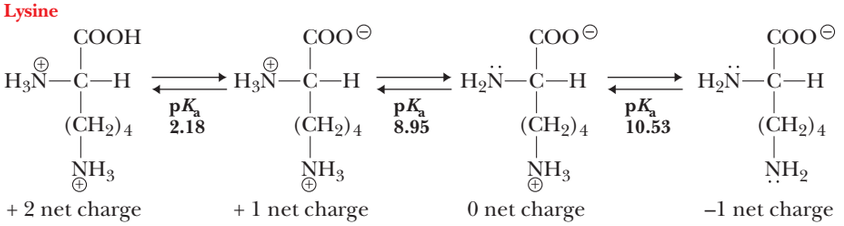
Histidine



Serine



Lysine



17. The pK_a for the ionization of the thiol group of cysteine is 8.33, so this amino acid could serve as a buffer in the —SH and S^{2-} forms over the pH range 7.33–9.33. The α-amino groups of asparagine and lysine have pK_a values of 8.80 and 8.95, respectively; these are also possible buffers, but they are both near the end of their buffer ranges.

18. At pH 4, the α-carboxyl group is deprotonated to a carboxylate, the side-chain carboxyl is still more than 50% protonated, and both amino groups are protonated. At pH 7, both the α-carboxyl group and the side-chain carboxyl group are deprotonated to a carboxylate, and both amino groups are protonated. At pH 10, both the α-carboxyl group and the side-chain carboxyl group are deprotonated to a carboxylate, one of the amino groups is primarily deprotonated, and the other amino group is a mixture of the protonated and deprotonated forms.

19. The pI refers to the form in which both carboxyl groups are deprotonated, and both amino groups protonated at pH 6.96.

20. At pH 1, the charged groups are the N-terminal NH_3^+ on valine and the protonated guanidino group on arginine, giving a net charge of +2. The charged groups at pH 7 are the same as those at pH 1, with the addition of the carboxylate group on the C-terminal leucine, giving a net charge of +1.

21. Both peptides, Phe—Glu—Ser—Met and Val—Trp—Cys—Leu, have a charge of +1 at pH 1 because of the protonated N-terminal amino group. At pH 7, the peptide on the right has no net charge because of the protonated

N-terminal amino group and the ionized C-terminal carboxylate negative charge. The peptide on the left has a net charge of −1 at pH 7 because of the side-chain carboxylate group on the glutamate in addition to the charges on the N-terminal and C-terminal groups.

22. (a) Lysine, because of the side-chain amino group.
    (b) Serine, because of the lack of a side-chain carboxyl.
23. Glycine is frequently used as the basis of a buffer in the acid range near the pK of its carboxyl group. The useful buffer range is pH 1.3–3.3.

3.4 The Peptide Bond

24. See Figure 3.10.
25. The resonance structures contribute to the planar arrangement by giving the CON bond partial double-bond character.
26. Tyrosine, tryptophan, and their derivatives.
27. A monoamine oxidase is an enzyme that degrades compounds with an amino group, including neurotransmitters; consequently, it can control a person's mental state.
28. The two peptides differ in amino acid sequence but not in composition.
29. The titration curves of the two peptides have the same general shape. The pK_a values of the α-amino and α-carboxyl groups differ. Very careful work will show slight differences in side-chain pK_a values because of the different distances to the charged groups at the ends of the peptide. Such changes are particularly marked in proteins.
30. Asp—Leu—Phe; Leu—Asp—Phe; Phe—Asp—Leu; Asp—Phe—Leu; Leu—Phe—Asp; Phe—Leu—Asp
31. DLF; LDF; FDL; DFL; LFD; FLD
32. You would get $20^{100} \approx 1.27 \times 10^{130}$ molecules, which is about 10^{84} Earth volumes. The same calculation for a pentapeptide gives more comprehensible results.
33. The different stereochemistry of the two peptides leads to different binding with taste receptors and therefore to the sweet taste for one and to the bitter taste for the other.
34. The high concentration of tryptophan in milk protein may mildly elevate the levels of serotonin, which relaxes the brain.
35. The tryptophan in milk might make you sleepy, whereas the tyramine in cheese should pep you up.
36. They are relatively stable because they are zwitterions. They typically have high melting points.
37. With very little doubt, no. Compare predicting the properties of water from those of hydrogen and oxygen, in either atomic or molecular form. If you knew the properties of the protein, you might be able to do the reverse to some extent.
38. The amino acids thyroxine and hydroxyproline occur in very few proteins. The genetic code does not include them, so they are produced by modification of tyrosine and proline, respectively.
39. These two peptides differ chemically. The open chain has a free C-terminal and N-terminal, but the cyclic peptide has only peptide bonds.
40. Both the C-terminal and the N-terminal of the open-chain peptide can be charged at appropriate pH values, which is not the case with the cyclic peptide. This can provide a basis for separation by electrophoresis.
41. Carbohydrates are not a source of the nitrogen needed for biosynthesis of amino acids.
42. Suggest that your friend show the carboxyl group as a charged carboxylate (—COO^-) and the amino group in its charged form (—NH_3^+).
43. Very few side chains have functional groups to form crosslinks.
44. Many more conformations would be possible because of free rotation around the peptide bond.
45. There would be no possibility of disulfide crosslinks within or between peptide chains, giving more possible conformations. There would not be the possibility of oxidation–reduction reactions involving sulfhydryl and disulfide groups.
46. The big difference would be the loss of stereospecificity in the conformation of any peptide or protein. This would have drastic consequences for the kinds of reactions of the protein.

3.5 Small Peptides with Physiological Activity

47. Oxytocin has an isoleucine at position 3 and a leucine at position 8; it stimulates smooth muscle contraction in the uterus during labor and in the mammary glands during lactation. Vasopressin has a phenylalanine at position 3 and an arginine at position 8; it stimulates resorption of water by the kidneys, thus raising blood pressure.
48. The reduced form of glutathione consists of three amino acids with a sulfhydryl group; the oxidized form consists of six amino acids and can be considered the result of linking two molecules of reduced glutathione by a disulfide bridge.
49. Enkephalins are pentapeptides (Y—G—G—F—L, leucine enkephalin, and Y—G—G—F—M, methionine enkephalin), which are naturally occurring analgesics.
50. In most cases, D-amino acids are toxic. They occur in nature in antibiotics and bacterial cell walls.

Chapter 4

4.1 Protein Structure and Function

1. (a) (iii); (b) (i); (c) (iv); (d) (ii).
2. When a protein is denatured, the interactions that determine secondary, tertiary, and any quaternary structures are overcome by the presence of the denaturing agent. Only the primary structure remains intact.
3. The random portions of a protein do not contain structural motifs that are repeated within the protein, such as α-helix or β-pleated sheet, but three-dimensional features of these parts of the protein are repeated from one molecule to another. Thus, the term *random* is somewhat of a misnomer.

4.2 Primary Structure of Proteins

4. When a protein is covalently modified, its primary structure is changed. The primary structure determines the final three-dimensional structure of the protein. The modification disrupts the folding process.
5. (a) Serine has a small side chain that can fit in any relatively polar environment.
   (b) Tryptophan has the largest side chain of any of the common amino acids, and it tends to require a nonpolar environment.
   (c) Lysine and arginine are both basic amino acids; exchanging one for the other would not affect the side-chain pK_a in a significant way. Similar reasoning applies to the substitution of a nonpolar isoleucine for a nonpolar leucine.
6. Glycine is frequently a conserved residue because its side chain is so small that it can fit into spaces that will not accommodate larger ones.
7. When alanine is replaced by isoleucine, there is not enough room in the native conformation for the larger side chain of the isoleucine. Consequently, there is a great enough change in the conformation of the protein that it loses activity. When glycine is substituted in turn for isoleucine, the presence of the smaller side chain leads to a restoration of the active conformation.
8. Meat consists largely of animal proteins and fat. The temperatures involved in cooking meat are usually more than enough to denature the protein portion of the meat.
9. Prion diseases have been linked to the immune system. It is believed that the prion proteins travel in the lymph system bound to lymphocytes and eventually arrive at the nervous tissue, where they begin to transform a normal cellular protein into an abnormal one (a prion).
10. Although there may be a strong genetic predisposition to acquire scrapie, that alone will not cause the disease. The disease must be started by ingesting a prion that already has the altered conformation, PrPsc.

4.3 Secondary Structure of Proteins

11. Shape, solubility, and type of biological function (static, structural versus dynamic, catalytic).
12. The protein efficiency ratio is an arbitrary measurement of the essential amino acid content of a given type of protein.
13. Eggs have the highest PER.
14. The essential amino acids are those that must be consumed in the diet because the body cannot synthesize them in sufficient quantities.
15. Reasons for creating genetically modified foods include increasing their protein content, increasing their shelf life, increasing their resistance to insects or other pests, and decreasing the need for using pesticides in order to grow them economically.
16. The angles of the amide planes as they rotate about the α-carbon. The angles are both defined as zero when the two planes would be overlapping such that the carbonyl group of one contacts the N—H of the other.

17. A β-bulge is a common nonrepetitive irregularity found in antiparallel β-sheets. A misalignment occurs between strands of the β-sheet, causing one side to bow outward.
18. A reverse turn is a region of a polypeptide where the direction changes by about 180°. There are two kinds—those that contain proline and those that do not. See Figure 4.6 for examples.
19. The α-helix is not fully extended, and its hydrogen bonds are parallel to the protein fiber. The β-pleated sheet structure is almost fully extended, and its hydrogen bonds are perpendicular to the protein fiber.
20. The αα unit, the βαβ unit, the β-meander, the Greek key, and the β-barrel.
21. The geometry of the proline residue is such that it does not fit into the α-helix, but it does fit exactly for a reverse turn. See Figure 4.10c.
22. Glycine is the only residue small enough to fit at crucial points in the collagen triple helix.
23. The principal component of wool is the protein keratin, which is a classic example of α-helical structure. The principal component of silk is the protein fibroin, which is a classic example of β-pleated sheet structure. The statement is somewhat of an oversimplification, but it is fundamentally valid.
24. Wool, which consists largely of the protein keratin, shrinks because of its α-helical conformation. It can stretch and then shrink. Silk consists largely of the protein fibroin, which has the fully extended β-sheet conformation, with far less tendency to stretch or shrink.

4.4 Tertiary Structure of Proteins

25. See Figure 4.2 for a hydrogen bond that is part of the α-helix (secondary structure). See Figure 4.13 for a hydrogen bond that is part of tertiary structure (side-chain hydrogen bonding).
26. See Figure 4.13 for electrostatic interactions, such as might be seen between the side chains of lysine and aspartate.
27. See Figure 4.13 for an example of a disulfide bond.
28. See Figure 4.13 for an example of hydrophobic bonds.
29. *Configuration* refers to the position of groups due to covalent bonding. Examples include *cis* and *trans* isomers and optical isomers. *Conformation* refers to the positioning of groups in space due to rotation around single bonds. An example is the difference between the eclipsed and staggered conformations of ethane.
30. Five possible features limit possible protein configurations and conformations. (1) Although any one of 20 amino acids is possible at each position, only one is used, as dictated by the gene that codes for that protein. (2) Either a D- or an L-amino acid could be used at each position (except for glycine), but only L-amino acids are used. (3) The peptide group is planar, so that only *cis* and *trans* arrangements are observed. The *trans* form is more stable and is the one usually found in proteins. (4) The angles ϕ and ψ can theoretically take on any value from 0° to 360°, but some angles are not possible because of steric hindrance; angles that are sterically allowed may not have stabilizing interactions, such as those in the α-helix. (5) The primary structure determines an optimum tertiary structure, according to the "second half of the genetic code."
31. Technically, collagen has quaternary structure because it has multiple polypeptide chains. However, most discussions of quaternary structure involve subunits of globular proteins, not fibrous ones like collagen. Many scientists consider the collagen triple helix to be an example of a secondary structure.

4.5 Quaternary Structure of Proteins

32. *Similarities:* both contain a heme group; both are oxygen binding; secondary structure is primarily α-helix. *Differences:* hemoglobin is a tetramer, while myoglobin is a monomer; oxygen binding to hemoglobin is cooperative, but noncooperative to myoglobin.
33. The crucial residues are histidines in both proteins.
34. Myoglobin's highest level of organization is tertiary. Hemoglobin's is quaternary.
35. The function of hemoglobin is oxygen transport; its sigmoidal binding curve reflects the fact that it can bind easily to oxygen at comparatively high pressures and release oxygen at lower pressures. The function of myoglobin is oxygen storage; as a result, it is easily saturated with oxygen at low pressures, as shown by its hyperbolic binding curve.
36. In the presence of H^+ and CO_2, both of which bind to hemoglobin, the oxygen-binding capacity of hemoglobin decreases.
37. In the absence of 2,3-*bis*phosphoglycerate, the binding of oxygen by hemoglobin resembles that of myoglobin, characterized by lack of cooperativity. 2,3-*Bis*phosphoglycerate binds at the center of the hemoglobin molecule, increases cooperativity, stabilizes the deoxy conformation of hemoglobin, and modulates the binding of oxygen so that it can easily be released in the capillaries.
38. Fetal hemoglobin binds oxygen more strongly than adult hemoglobin. See Figure 4.25.
39. Histidine 143 in a β-chain is replaced by a serine in a γ-chain.
40. Deoxygenated hemoglobin is a weaker acid (has a higher pK_a) than oxygenated hemoglobin. In other words, deoxygenated hemoglobin binds more strongly to H^+ than does oxygenated hemoglobin. The binding of H^+ (and of CO_2) to hemoglobin favors the change in quaternary structure to the deoxygenated form of hemoglobin.
41. The primary flaw in your friend's reasoning is a reversal of the definition of pH, which is pH = –log [H^+]. If the release or binding of hydrogen ion by hemoglobin were the primary factor in the Bohr effect, the pH changes would be the opposite of those actually observed. The response of hemoglobin to changes in pH is the central point. When the pH increases, the hydrogen ion concentration decreases, and vice versa.
42. The change of a histidine to a serine in the γ-chain removes a positively charged amino acid that could have interacted with BPG. Thus there are fewer salt bridges to break, so binding is easier than it is in a β-chain.
43. People with sickle-cell trait have some abnormal hemoglobin. At high altitudes, there is less oxygen, and the concentration of the deoxy form of the abnormal hemoglobin increases. Less oxygen can be bound, causing the observed breathing difficulties.
44. In fetal hemoglobin, the subunit composition is $\alpha_2\gamma_2$ with replacement of the β-chains by the γ-chains. The sickle-cell mutation affects the β-chain, so the fetus homozygous for Hb S has normal fetal hemoglobin.
45. The relative oxygen affinities allow oxygen to be taken by the fetal cells from the maternal Hb.
46. Because people with sickle-cell disease are chronically anemic, some cells with fetal Hb are produced to help overcome the impaired oxygen delivery system.
47. The crystalline form changed because oxygen entered under the cover slip, transforming deoxyhemoglobin to oxyhemoglobin.

4.6 Protein Folding Dynamics

48. This level of sequence homology is marginal for use of comparative modeling. It is best to try that method, but then to compare the results with those obtained from the fold-recognition approach.
49. Protein folding is driven by many processes. The intuitive ones are the direct interactions of functional groups through covalent bonds, electrostatic attractions, and hydrogen bonds. These explain why parts of the protein are attracted to each other and why a protein would tend to adopt a shape making these interactions possible. However, much of the protein-folding process is also driven by an entropy effect. We refer to hydrophobic interactions as an explanation of why nonpolar regions of the protein tend to cluster together, usually in the interior of the protein. However, in reality, it is not the interaction of nonpolar amino acids that drives this process. It is actually the increase in entropy of the solvent, water. When the hydrophobic regions of the protein are isolated to the interior, the water molecules surrounding the protein are more free to rotate and move in less restricted ways. Thus, what drives much of protein folding is not a ΔH change with the bonding of specific amino acids, but rather a ΔS increase of the solvent.
50. See the Protein Data Bank.
51. A chaperone is a protein that aids another protein in folding correctly and keeps it from associating with other proteins before it has reached its final, mature form.
52. A prion is a potentially infectious protein found in multiple forms in mammals, often concentrated in nervous tissue. It is an abnormal form of a normal cellular protein. It tends to form plaques that destroy the nervous tissue. Prions have been found to be transmissible across species.
53. A series of encephalopathies have been found to be caused by prions. In cows, the disease caused by prions is called bovine spongiform encephalopathy, or more commonly mad-cow disease. In sheep, the disease is called scrapie. In humans, it is called Creutzfeldt-Jakob disease.
54. The normal form of the prion protein has a higher α-helix content compared to the β-sheet content. The abnormal one has an increased β-sheet content.
55. Alzheimer's, Parkinson's, and Huntington's diseases are caused by accumulation of protein deposits from aggregates caused by misfolded proteins. This chapter also looked at prion diseases. When prions are misfolded they

can cause spongiform encephalopathies, such as mad-cow disease, and the human form, Creutzfeldt-Jakob disease.

56. Protein aggregates form when there are exposed areas on a protein surface that are nonpolar. Proteins then stick together via these nonpolar regions causing the aggregates. An example is the prion disease in which an area of the normal molecule that should be an α-helix adopts a β-sheet conformation instead.
57. The root problem with the globin genes and potential issues with hemoglobin formation is based on the fact that there are two α-globin genes for every β-globin gene, yet to make hemoglobin they must combine in a 1:1 ratio. Thus, one theoretically possible solution would be if there were not a 2:1 ratio of these genes. Another issue is that the two genes are on different chromosomes. They are then most likely controlled separately. If the two genes were close together on one chromosome, then they could be controlled together by the same signal and produced in the correct amounts.
58. The sequence of the mutant prion that confers the most extreme sensitivity to conferring a prion disease is the substitution of the amino acid at position 129 to a methionine.
59. Any disease with a long incubation time is potentially more serious than many would recognize. After the initial determination that prion diseases could jump species and the BSE scare that happened in the UK in the late 1990s, researchers are worried that there may still be a reservoir of human beings carrying a latent prion disease that we have not seen develop yet. The same problem could afflict cattle and sheep. The fact that no pandemic has happened yet is not a guarantee that there will not be one.
60. The spongiform encephalopathies that we know of have characteristics of both inherited diseases and transmissible diseases. On the one hand, animals can be infected by consuming meat or other tissues that are themselves carrying mutant prion proteins. As the example of the New Zealand sheep showed, even those that are very susceptible to a prion disease can remain disease-free if they are never exposed. However, the predisposition to acquire a prion disease has a hereditary component as well. The prion protein has many known mutations, some of which render the individual very susceptible to the disease. These mutations can be tracked and they are passed along family lines.

Chapter 5

5.1 Extracting Pure Proteins from Cells

1. Using a blender, a Potter-Elvejhem homogenizer, or a sonicator.
2. If you needed to maintain the structural integrity of the subcellular organelles, a Potter-Elvejhem homogenizer would be better because it is more gentle. The tissue, such as liver, must be soft enough to use with this device.
3. Salting out is a process whereby a highly ionic salt is used to reduce the solubility of a protein until it comes out of solution and can be centrifuged. The salt forms ion–dipole bonds with the water in the solution, which leaves less water available to hydrate the protein. Nonpolar side chains begin to interact between protein molecules, and they become insoluble.
4. Their amino acid content and arrangements make some proteins more soluble than others. A protein with more highly polar amino acids on the surface is more soluble than one with more hydrophobic ones on the surface.
5. First homogenize the liver cells using a Potter-Elvejhem homogenizer. Then spin the homogenate at 500 × *g* to sediment the unbroken cells and nuclei. Centrifuge the supernatant at 15,000 × *g* and collect the pellet, which contains the mitochondria.
6. No, peroxisomes and mitochondria have overlapping sedimentation characteristics. Other techniques, such as sucrose-gradient centrifugation, would have to be used to separate the two organelles.
7. If the protein were cytosolic, once the cells were broken open, you could centrifuge at 100,000 × *g*, and all the organelles would be in the pellet. Your enzyme would be in the supernatant, along with all the other cytosolic ones.
8. Isolate the mitochondria via differential or sucrose-gradient centrifugation. Use another homogenization technique, combined with a strong detergent, to release the enzyme from the membrane.
9. Tables exist to tell you how many grams of ammonium sulfate $[(NH_4)_2SO_4]$ to add to get a certain percent saturation. A good plan would be to take the homogenate and add enough ammonium sulfate to yield a 20% saturated solution. Let the sample sit for 15 minutes on ice and then centrifuge. Separate the supernatant from the precipitate. Assay both for the protein you are working with. Add more ammonium sulfate to the supernatant to arrive at a 40% saturated solution and repeat the process. In this way, you will find out what the percent saturation in ammonium sulfate needs to be to precipitate the protein.
10. Reasonably harsh homogenization would be able to liberate the soluble protein X from the peroxisomes, which are fragile. Centrifugation at 15,000 × *g* would sediment the mitochondria (broken or intact). The supernatant would then have protein X but no protein Y. Freeze/thaw techniques and sonication would accomplish the same thing, or the mitochondria and the peroxisomes could be separated initially by sucrose-gradient centrifugation.

5.2 Column Chromatography

11. (a) Size.
    (b) Specific ligand-binding ability.
    (c) Net charge.
12. The largest proteins elute first; the smallest elute last. Larger proteins are excluded from the interior of the gel bead so they have less available column space to travel. Essentially, they travel a shorter distance and elute first.
13. A compound can be eluted by raising the salt concentration or by adding a mobile ligand that has a higher affinity for the bound protein than the stationary resin ligand does. Salt is cheaper but less specific. A specific ligand may be more specific, but it is likely to be expensive.
14. A compound can be eluted by raising the salt concentration or by changing the pH. Salt is cheap, but it might not be as specific for a particular protein. Changing the pH may be more specific for a tight pI range, but extremes of pH may also denature the protein.
15. Raising the salt concentration is relatively safe. Most proteins will elute this way, and, if the protein is an enzyme, it will still be active. If necessary, the salt can be removed later via dialysis. Changing the pH enough to remove the charge can cause the proteins to become denatured. Many proteins are not soluble at the isoelectric points.
16. The basis of most resins is agarose, cellulose, dextran, or polyacrylamide.
17. See Figure 5.7.
18. Within the fractionation range of a gel-filtration column, molecules elute with a linear relationship of log MW versus their elution volumes. A series of standards can be run to standardize the column, and then an unknown can be determined by measuring its elution volume and comparing it to a standard curve.
19. Both proteins would elute in the void volume together and would not be separated.
20. Yes, the β-amylase would come out in the void volume, but the bovine serum albumin would be included in the column bead and would elute more slowly.
21. Set up an anion-exchange column, such as Q-Sepharose (quaternary amine). Run the column at pH 8.5, a pH at which the protein X has a net negative charge. Put a homogenate containing protein X on the column and wash with the starting buffer. Protein X will bind to the column. Then elute by running a salt gradient.
22. Use a cation-exchange column, such as CM-Sepharose, and run it at pH 6. Protein X will have a positive charge and will stick to the column.
23. With a quaternary amine, the column resin always has a net positive charge, and you don't have to worry about the pH of your buffer altering the form of the column. With a tertiary amine, there is a dissociable hydrogen, and the resin may be positive or neutrally charged, depending on the buffer pH.
24. The easiest way would be to use a sucrose gradient to separate the mitochondria from the peroxisomes first. Then break open the mitochondria via harsh homogenization or sonication, and then centrifuge the mitochondria. The pellet would contain protein B, while the supernatant would contain protein A. Contaminants could still exist, but they could be cleaned away by running gel filtration, on Sephadex G-75 (which would separate enzyme C from enzymes A and B), and then by running ion-exchange chromatography on Q-Sepharose at pH 7.5. Enzyme B would be neutral and would elute, while enzyme A would stick to the column.
25. Glutamic acid will be eluted first because the column pH is close to its pI. Leucine and lysine will be positively charged and will stick to the column. To elute leucine, raise the pH to around 6. To elute lysine, raise the pH to around 11.
26. A nonpolar mobile solvent will move the nonpolar amino acids fastest, so phenylalanine will be the first to elute, followed by glycine and then glutamic acid.

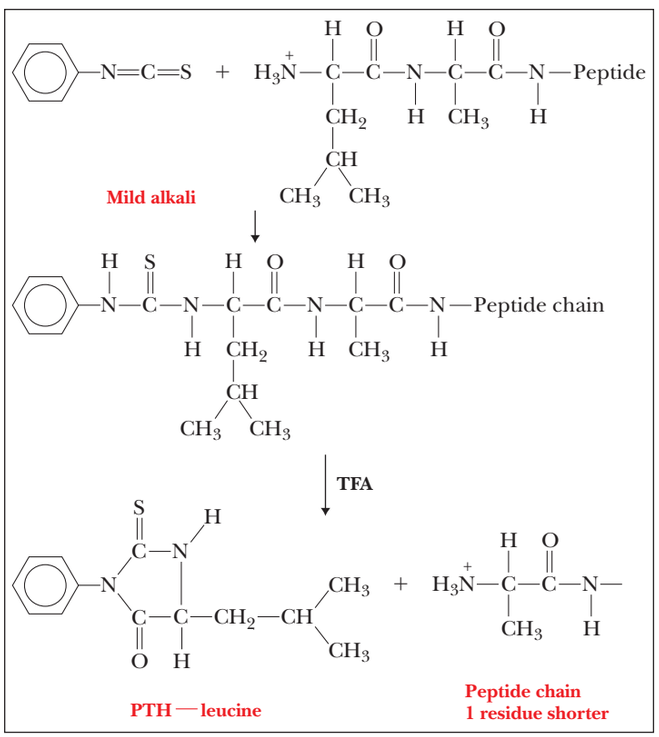
27. The nonpolar amino acids will stick the most to the stationary phase, so glutamic acid will move the fastest, followed by glycine and then phenylalanine.
28. A protein solution from an ammonium sulfate preparation is passed over a gel-filtration column where the proteins of interest will elute in the void volume. The salt, being very small, will move through the column slowly. In this way, the proteins will leave the salt behind and exit the column without it.

5.3 Electrophoresis

29. Size, shape, and charge.
30. Agarose and polyacrylamide.
31. Polyacrylamide.
32. DNA is the molecule most often separated on agarose electrophoresis, although proteins can also be separated.
33. Those with the highest charge/mass ratio would move the fastest. There are three variables to consider, and most electrophoreses are done in a way to eliminate two of the variables so that the separation is by size or by charge, but not by both.
34. Sodium dodecyl-sulfate polyacrylamide gel-electrophoresis. With SDS–PAGE, the charge and shape differences of proteins are eliminated so that the only parameter determining the migration is the size of the protein.
35. SDS binds to the protein in a constant ratio of 1.4 g SDS per gram of protein. It coats the protein with negative charges and puts it into a random coil shape. Thus, charge and shape are eliminated.
36. In a polyacrylamide gel used for gel-filtration chromatography, the larger proteins can travel around the beads, thereby having a shorter path to travel and therefore eluting faster. With electrophoresis, the proteins are forced to go through the matrix, so the larger ones travel more slowly because there is more friction.
37. The MW is 37,000 Da.

5.4 Determining the Primary Structure of a Protein

38. The Edman degradation will give the identity of the N-terminal amino acid in its first cycle, so doing a separate experiment is not necessary.
39. It might tell you if the protein were pure or if there were subunits.
40.



41. The amount of Edman reagent must exactly match the amount of N-termini in the first reaction. If there is too little Edman reagent, some of the N-termini will not react. If there is too much, some of the second amino acid will react. In either case, there will be a small amount of contaminating phenylthiohydantoin (PTH) derivatives. This error grows with the number of cycles run until the point that two amino acids are released in equal amounts, and you cannot tell which one was supposed to be the correct one.
42. In the first cycle, the first and second amino acids from the N-terminal end would be reacted and released as PTH derivatives. You would get a double signal and not know which one was the true N-terminus.
43. Val—Leu—Gly—Met—Ser—Arg—Asn—Thr—Trp—Met—Ile—Lys—Gly—Tyr—Met—Gln—Phe
44. Met—Val—Ser—Thr—Lys—Leu—Phe—Asn—Glu—Ser—Arg—Val—Ile—Trp—Thr—Leu—Met—Ile
45. It is possible that your protein is not pure and needs additional purification steps to arrive at a single polypeptide. It is also possible that the protein has subunits, so multiple polypeptide chains could be yielding the contradictory results.
46. There are two fragments that have C-termini that are not lysine or arginine, which is what trypsin is specific for. Normally there would be only one fragment ending with an amino acid that was not Arg or Lys, and we would immediately know that it was the C-terminus. Histidine is a basic amino acid, although it is usually neutral and therefore does not react with trypsin. It is possible that, in the pH environment of the reaction, the histidine was positively charged and was recognized by trypsin.
47. It would tell you a relative concentration of the various amino acids. This is important because it would help you plan your sequencing experiment better. For example, if you had a protein whose composition showed no aromatic amino acids, it would be a waste of time to use a chymotrypsin digestion.
48. Cyanogen bromide would be useless, because there is no methionine. Trypsin would be little better, because the protein is 35% basic residues. Trypsin would shred the protein into more than 30 pieces, which would be very hard to analyze.
49. Chymotrypsin would be a good choice. There are more than four residues of aromatic amino acids. The protein, containing 100 amino acids, would be cut four times, possibly yielding nice fragments roughly 20–30 amino acids long, which can be sequenced effectively by the Edman degradation.
50. It would work best if the basic residues were spread out in the protein. In that way, fragments in the proper size range would be generated. If all four of the basic residues were in the first 10 amino acids, there would be one long fragment that could not be sequenced.
51. Proteomics is the systematic analysis of an organism's complete complement of proteins, or its **proteome.** Just as we learned the basic dogma of molecular biology (DNA → RNA → protein), the technology now available has allowed scientists to describe all the DNA of an organism as its genome, all of the RNA as its transcriptome, and all of the proteins produced as its proteome. To understand the flux of proteins in a cell is to understand its metabolism.
52. The bait protein is constructed to have a particular affinity tag. The bait protein interacts with cell proteins of interest and then binds to an affinity column via the tag. In this way, the cell proteins of interest can be found and isolated.
53. There are many assumptions behind the experiment described in the Biochemical Connections on page 139. One must assume that the nature of the tag has not changed the binding of the protein. For example, if adding the tag makes a protein more likely or less likely to bind to it, then the conclusions about cellular protein binding may be incorrect. For example, one might conclude that two proteins bind together to serve their metabolic function, but this binding could be an artifact of the experimental conditions. One must also assume that tagging the proteins has not changed the affinity between the tag and the affinity column.

Chapter 6

6.1 Enzymes Are Effective Biological Catalysts

1. Enzymes are many orders of magnitude more effective as catalysts than are nonenzymatic catalysts.
2. Most enzymes are proteins, but some catalytic RNAs (ribozymes) are known.
3. About 3 seconds (1 year × 1 event/10^7 events × 365 days/year × 24 hours/day × 3600 seconds/hour = 3.15 seconds).
4. Enzymes hold the substrates in favorable spatial positions, and they bind effectively to the transition state to stabilize it. Note that *all* catalysts lower the activation energy, so this is not a particular enzyme function.

6.2 Kinetics versus Thermodynamics

5. The reaction of glucose with oxygen is thermodynamically favored, as shown by the negative free-energy change. The fact that glucose can be maintained

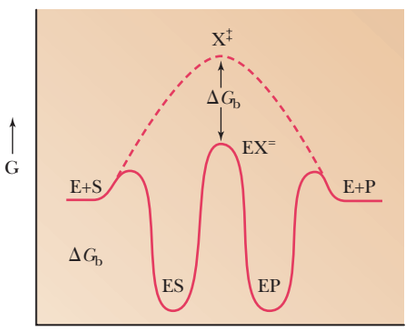
in an oxygen atmosphere is a reflection of the kinetic aspects of the reaction, requiring overcoming an activation-energy barrier.

6. To the first question, most probably: local concentrations (mass-action concepts) could easily dictate the direction. To the second question, probably not: local concentrations would seldom be sufficient to overcome a relatively large ΔG° of –5.3 kcal in the reverse reaction. (See, however, the aldolase reaction in glycolysis.)
7. Heating a protein denatures it. Enzymatic activity depends on the correct three-dimensional structure of the protein. The presence of bound substrate can make the protein harder to denature.
8. The results do not prove that the mechanism is correct because results from different experiments could contradict the proposed mechanism. In that case, the mechanism would have to be modified to accommodate the new experimental results.
9. The presence of a catalyst affects the rate of a reaction. The standard free-energy change is a thermodynamic property that does not depend on the reaction rate. Consequently, the presence of the catalyst has no effect.
10. The presence of a catalyst lowers the activation energy of a reaction.
11. Enzymes, like all catalysts, increase the rate of the forward and reverse reaction to the same extent.
12. The amount of product obtained in a reaction depends on the equilibrium constant. A catalyst does not affect that.

6.3 Enzyme Kinetic Equations

13. The reaction is first order with respect to A, first order with respect to B, and second order overall. The detailed mechanism of the reaction is likely to involve one molecule each of A and B.
14. The easiest way to follow the rate of this reaction is to monitor the decrease in absorbance at 340 nm, reflecting the disappearance of NADH.
15. The use of a pH meter would not be a good way to monitor the rate of the reaction. You are probably running this reaction in a buffer solution to keep the pH relatively constant. If you are not running the reaction in a buffer solution, you run the risk of acid denaturation of the enzyme.
16. Enzymes tend to have fairly sharp pH optimum values. It is necessary to ensure that the pH of the reaction mixture stays at the optimum value. This is especially true for reactions that require or produce hydrogen ions.

6.4 Enzyme–Substrate Binding

17. In the lock-and-key model, the substrate fits into a comparatively rigid protein that has an active site with a well-defined shape. In the induced-fit model, the enzyme undergoes a conformational change on binding to the substrate. The active site takes shape around the substrate.
18. 

No destabilization, thus no catalysis.

No destabilization, thus no catalysis.

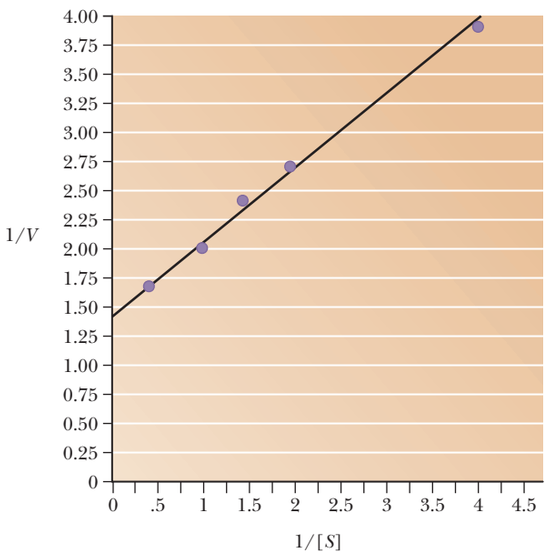
19. The ES complex would be in an "energy trough," with a consequentially large activation energy to the transition state.
20. Amino acids that are far apart in the amino acid sequence can be close to each other in three dimensions because of protein folding. The critical amino acids are in the active site.
21. The overall protein structure is needed to ensure the correct arrangement of amino acids in the active site.
22. The strong inhibition indicates tight binding to the active site. Thus, the compound is very likely to be a transition-state analogue.

6.5 Examples of Enzyme-Catalyzed Reactions

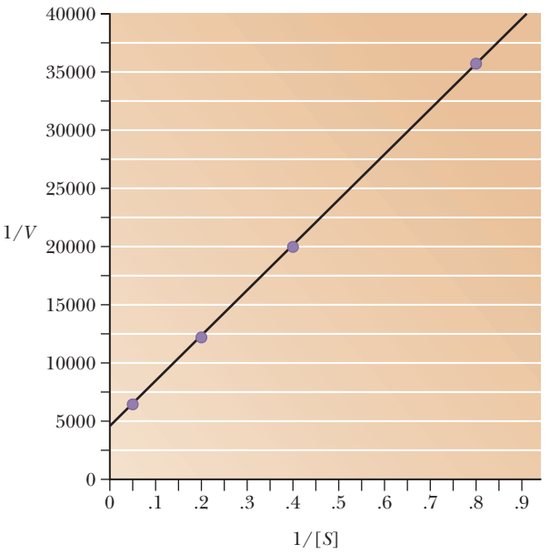
23. See Figures 6.6 and 6.7.
24. Not all enzymes follow Michaelis–Menten kinetics. The kinetic behavior of allosteric enzymes does not obey the Michaelis–Menten equation.
25. The graph of rate against substrate concentration is sigmoidal for an allosteric enzyme but hyperbolic for an enzyme that obeys the Michaelis–Menten equation.

6.6 The Michaelis–Menten Approach to Enzyme Kinetics

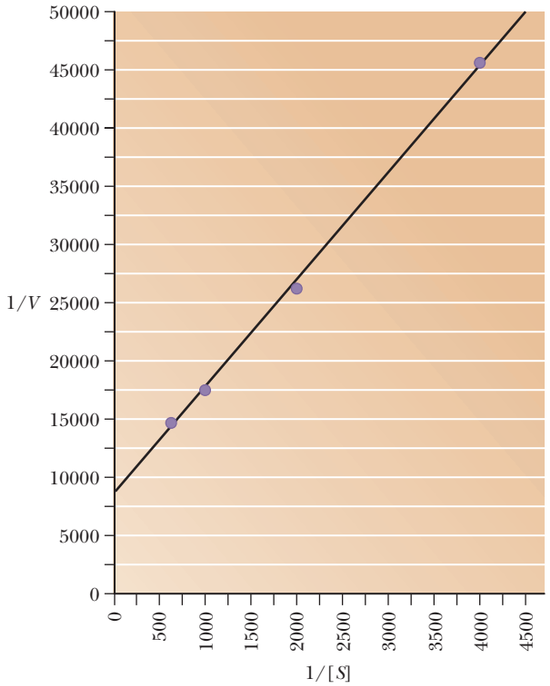
26. The reaction velocity remains the same with increasing enzyme concentration. It is theoretically possible, but highly unlikely, for a reaction to be saturated with enzyme.
27. The steady-state assumption is that the concentration of the enzyme–substrate complex does not change appreciably over the time in which the experiment takes place. The rate of appearance of the complex is set equal to its rate of disappearance, simplifying the equations for enzyme kinetics.
28. Turnover number = $V_{max}/[ET]$.
29. Use Equation 6.16.
    (a) $V = 0.5V_{max}$
    (b) $V = 0.33V_{max}$
    (c) $V = 0.09V_{max}$
    (d) $V = 0.67V_{max}$
    (e) $V = 0.91V_{max}$
30. See graph: $V_{max} = 0.681$ mM min^{-1}, $K_M = 0.421$ M.



31. See graph: $V_{max} = 2.5 \times 10^{-4}$ M sec^{-1}, $K_M = 1.6 \times 10^{8}$ M.



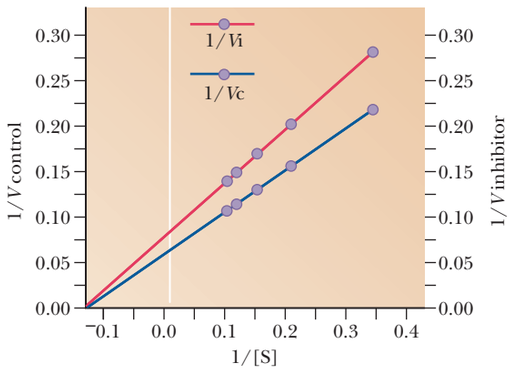
32. See graph: $K_M = 2.86 \times 10^{-2}$ *M*. Concentrations were not determined directly. Absorbance values were used instead as a matter of convenience.
33. See graph: $V_{max} = 1.32 \times 10^{-3}$ *M* min^{-1}, $K_M = 1.23 \times 10^{-3}$ *M*.



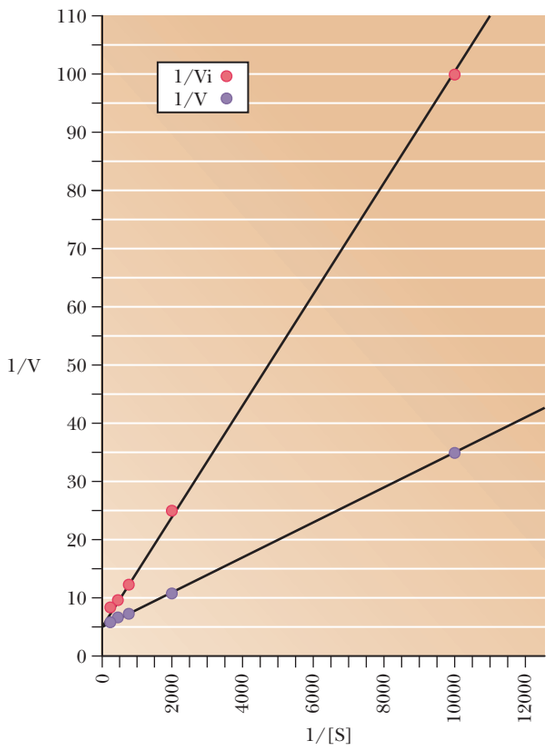
34. The turnover number is 20.43 min^{-1}.
35. The number of moles of enzyme is 1.56×10^{-10}. The turnover number is 10,700 sec^{-1}.
36. The low K_M for the aromatic amino acids indicates that they will be oxidized preferentially.
37. It is easier to detect deviations of individual points from a straight line than from a curve.
38. The assumption that the K_M is an indication of the binding affinity between the substrate and the enzyme is valid when the rate of dissociation of the enzyme–substrate complex to product and enzyme is much smaller than the rate of dissociation of the complex to enzyme and substrate.

6.7 Enzyme Inhibition

39. In the case of competitive inhibition, the value of K_M increases, while the value of K_M remains unchanged in noncompetitive inhibition.
40. A competitive inhibitor blocks binding, not catalysis.
41. A noncompetitive inhibitor does not change the affinity of the enzyme for its substrate.
42. A competitive inhibitor binds to the active site of an enzyme, preventing binding of the substrate. A noncompetitive inhibitor binds at a site different from the active site, causing a conformational change, which renders the active site less able to bind substrate and convert it to product.
43. Competitive inhibition can be overcome by adding enough substrate, but this is not true for all forms of enzyme inhibition.
44. A Lineweaver–Burk plot is useful because it gives a straight line. It is easier to determine how well points fit to a straight line than to a curve.
45. In a Lineweaver–Burk plot for competitive inhibition, the lines intersect at the *y*-axis intercept, which is equal to $1/V_{max}$. In a Lineweaver–Burk plot for noncompetitive inhibition, the lines intersect at the *x*-axis intercept, which is equal to $-1/K_M$.
46. $K_M = 7.42$ m*M*; $V_{max} = 15.9$ mmol min^{-1}; noncompetitive inhibition.



47. Competitive inhibition, $K_M = 6.5 \times 10^{-4}$. The key point here is that the V_{max} is the same within the limits of error. Some of the concentrations are given to one significant figure.



48. It is *very* good, in the case of noncompetitive inhibitors; much of metabolic control depends on feedback inhibition by downstream noncompetitive inhibitors. The question is perhaps moot in the case of competitive inhibitors, which are much less commonly encountered in vivo. Some antibiotics, however, are competitive inhibitors (good for the sick person, bad for the bacteria).
49. Both the slope and the intercepts will change. The lines will intersect above the *x*-axis at negative values of $1/[S]$.
50. Not all AIDS drugs are enzyme inhibitors, but an important class of such drugs inhibits the HIV protease. You would need to understand the concepts of substrate binding, inhibition, and inhibitor binding.
51. An irreversible inhibitor is bound by covalent bonds. Noncovalent interactions are relatively weak and easily broken.
52. A noncompetitive inhibitor does not bind to the active site of an enzyme. Its structure need bear no relation to that of the substrate.

Chapter 7

7.1 The Behavior of Allosteric Enzymes

1. Allosteric enzymes display sigmoidal kinetics when rates are plotted versus substrate concentration. Michaelis–Menten enzymes exhibit hyperbolic kinetics. Allosteric enzymes usually have multiple subunits, and the binding of substrates or effector molecules to one subunit changes the binding behavior of the other subunits.
2. It is an enzyme used in the early stages of cytidine nucleotide synthesis.
3. ATP acts as a positive effector of ATCase, and CTP acts as an inhibitor.
4. The term K_M should be used for enzymes that display Michaelis–Menten kinetics. Thus, it is not used with allosteric enzymes. Technically, competitive and noncompetitive inhibition are also terms that are restricted to Michaelis–Menten enzymes, although the concepts are applicable to any enzyme. An inhibitor that binds to an allosteric enzyme at the same site as the substrate is similar to a classical competitive inhibitor. One that binds at a different site is similar to a noncompetitive inhibitor, but the equations and the graphs characteristic of competitive and noncompetitive inhibition don't work the same way with an allosteric enzyme.
5. A K system is an allosteric enzyme in which the binding of inhibitor alters the apparent substrate concentration needed to reach one-half V_{max}, $S_{0.5}$.
6. A V system is an allosteric enzyme in which the binding of inhibitor changes the V_{max} of the enzyme but not the $S_{0.5}$.
7. Homotropic effects are allosteric interactions that occur when several identical molecules are bound to a protein. The binding of substrate molecules to different sites on an enzyme, such as the binding of aspartate to ATCase, is an example of a homotropic effect. Heterotropic effects are allosteric interactions that occur when different substances (such as inhibitor and substrate) are bound to the protein. In the ATCase reaction, inhibition by CTP and activation by ATP are both heterotropic effects.
8. ATCase is made up of two different types of subunits. One of them is the catalytic subunit, and there are six of them organized into two trimers. The other is the regulatory subunit, which consists of six protein subunits organized into three dimers.
9. Enzymes that exhibit cooperativity do not show hyperbolic curves of rate versus substrate concentration. Their curves are sigmoidal. The level of cooperativity can be seen by the shape of the sigmoidal curve.
10. Inhibitors make the shape of the curve more sigmoidal.
11. Activators make the shape of the curve less sigmoidal.
12. $K_{0.5}$ is the substrate concentration that leads to half of the maximal velocity. This term is used with allosteric enzymes, where the term K_M is not appropriate.
13. A mercury compound was used to separate the subunits of ATCase. When the subunits were separated, one type of subunit retained catalytic activity but was no longer allosteric and was not inhibited by CTP. The other subunit type had no ATCase activity, but it did bind to CTP and ATP.

7.2 The Concerted and Sequential Models for Allosteric Enzymes

14. In the concerted model, all the subunits in an allosteric enzyme are found in the same form, either the T form or the R form. They are in equilibrium, with each enzyme having a characteristic ratio of the T/R. In the sequential model, the subunits change individually from T to R.
15. The sequential model can explain negative cooperativity, because a substrate binding to the T form could induce other subunits to switch to the T form, thereby reducing binding affinity.
16. Greater cooperativity is favored by having a higher ratio of the T/R form. It is also favored by having a higher dissociation constant for the substrate binding to the T form.
17. The L value is the equilibrium ratio of the T/R form. The c value is the ratio of the dissociation constants for substrate and the two forms of enzyme, such that $c = K_R/K_T$.
18. Many models are possible. We never really know for sure how the enzyme works, rather, we create a model that explains the observed behavior. It is very possible that another model would do so as well.

7.3 Control of Enzyme Activity by Phosphorylation

19. A kinase is an enzyme that phosphorylates a protein using a high-energy phosphate, such as ATP, as the phosphate donor.
20. Serine, threonine, and tyrosine are the three most often phosphorylated amino acids in proteins that are acted upon by kinases. Aspartate is another one often phosphorylated.
21. The allosteric effect can be faster because it is based on simple binding equilibrium. For example, if AMP is an allosteric activator of glycogen phosphorylase, the immediate increase in AMP when muscles contract can cause muscle phosphorylase to become more active and to provide energy for the contracting muscles. The phosphorylation effect requires the hormone cascade beginning with glucagon or epinephrine. There are many steps before the glycogen phosphorylase is phosphorylated, so the response time is slower. However, the cascade effect produces many more activated phosphorylase molecules, so the effects are longer and stronger.
22. As part of the mechanism, the sodium–potassium ATPase has an aspartate residue that becomes phosphorylated. This phosphorylation alters the conformation of the enzyme and causes it to close on one side of the membrane and open on the other, moving ions in the process.
23. Glycogen phosphorylase is controlled allosterically by several molecules. In the muscle, AMP is an allosteric activator. In the liver, glucose is an allosteric inhibitor. Glycogen phosphorylase also exists in a phosphorylated form and an unphosphorylated form, with the phosphorylated form being more active.

7.4 Zymogens

24. The digestive enzymes trypsin and chymotrypsin are classic examples of regulation by zymogens. The blood-clotting protein thrombin is another.
25. Trypsin, chymotrypsin, and thrombin are all proteases. Trypsin cleaves peptide bonds where there are amino acids with positively charged side chains (Lys and Arg). Chymotrypsin cleaves peptides at amino acids with aromatic side chains. Thrombin cleaves the protein fibrinogen into fibrin.
26. The zymogen prothrombin is cleaved to give the active enzyme thrombin. The thrombin then cleaves a soluble molecule, fibrinogen, into an insoluble molecule, fibrin. Fibrin is a protein that forms part of the blood clot.
27. Chymotrypsinogen is an inactive zymogen. It is acted upon by trypsin, which cleaves peptides at basic residues, like arginine. When trypsin cleaves between the arginine and the isoleucine, chymotrypsinogen becomes semiactive, forming π-chymotrypsin. This molecule digests itself further, forming the active α-chymotrypsin. As it turns out, the α-amino group of the isoleucine produced by the first cleavage is near the active site of α-chymotrypsin and necessary for its activity.
28. Zymogens are often seen with digestive enzymes that are produced in one tissue and used in another. If the enzyme were active immediately upon production, it would digest other cell proteins, where it would cause great damage. By having it produced as a zymogen, it can be safely made and then transported to the digestive tissue, such as the stomach or small intestine, where it can then be activated.
29. This allows for a more rapid response when the hormone is needed. The hormone is already synthesized and usually just requires breaking one or two bonds to make it active. The hormone can be poised and ready to go on demand.

7.5 The Nature of the Active Site

30. Serine and histidine are the two most critical amino acids in the active site of chymotrypsin.
31. The initial phase releases the first product and involves an acylenzyme intermediate. This step is faster than the second part, in which water comes into the active site and breaks the acyl-enzyme bond.
32. In the first step of the reaction, the serine hydroxyl is the nucleophile that attacks the substrate peptide bond. In the second step, water is the nucleophile that attacks the acyl-enzyme intermediate.
33. Histidine 57 performs a series of steps involving general base catalysis followed by general acid catalysis. In the first phase, it takes a hydrogen from serine 195, acting as a general base. This is followed immediately by an acid catalysis step, in which it gives the hydrogen to the amide group of the peptide bond that is breaking. A similar scheme takes place in the second phase of the reaction.
34. The first phase is faster for several reasons. The serine at position 195 is a strong nucleophile for the initial nucleophilic attack. It then forms an acyl-enzyme intermediate. In the second phase, water is the nucleophile, and it takes time for water to diffuse to the right spot to perform its nucleophilic attack. It is also not as strong a nucleophile as the serine. Therefore, it takes longer for water to perform its nucleophilic attack and break the acyl-enzyme intermediate than it takes for serine to create it.
35. Histidine 57 exists in both the protonated and unprotonated form during the chymotrypsin reaction. Its pK_a of 6.0 makes this possible in the physiological pH range.

36. Instead of a phenylalanine moiety (similar to the usual substrates of chymotrypsin), use a nitrogen-containing basic group similar to the usual substrates of trypsin.

7.6 Chemical Reactions Involved in Enzyme Mechanisms

37. They act as Lewis acids (electron-pair acceptors) and can take part in enzyme catalysis mechanisms of enzymes.
38. False. The mechanisms of enzymatic catalysis are the same as those encountered in organic chemistry, operating in a complex environment.
39. General acid catalysis is the part of an enzyme mechanism in which an amino acid or other molecule donates a hydrogen ion to another molecule.
40. S_N1 stands for unimolecular nucleophilic substitution. The unimolecular part means that it obeys first-order kinetics. If the reaction is R:X + Z: → R:Z + X:, with an S_N1 reaction, the rate depends on the speed with which the X breaks away from the R. The Z group comes in later and quickly, compared with the breakdown of R:X. S_N2 stands for bimolecular nucleophilic substitution. This happens with the same reaction scheme if the Z attacks the R:X molecule before it breaks down. Thus, the concentration of both R:X and Z: are important, and the rate displays second-order kinetics.
41. The S_N1 reaction leads to loss of stereospecificity as the X group leaves before the entering nucleophile. This means that the nucleophile can enter from different angles, leading to different isomers.
42. The results do not prove that the mechanism is correct, because results from different experiments could contradict the proposed mechanism. In that case, the mechanism would have to be modified to accommodate the new experimental results.

7.7 The Active Site and Transition States

43. A good transition-state analogue would have to have a tetrahedral carbon atom where the amide carbonyl group was originally found, since the transition state involves a momentary tetrahedral form. It would also have to have oxygens on the same carbon, so that there would be sufficient specificity for the active site.
44. The induced-fit model assumes that the enzyme and substrate must both move and change to conform to each other perfectly. Thus, the true fit is not between the enzyme and substrate but between the enzyme and the transition state of the substrate on its way to product. A transition-state analogue fits the enzyme nicely in this model.
45. An abzyme is created by injecting a host animal with a transition-state analogue of a reaction of interest. The host animal makes antibodies to the foreign molecule, and these antibodies have specific binding points that mimic an enzyme surrounding a transition state. The purpose is to create an antibody with catalytic activity.
46. Cocaine blocks the reuptake of the neurotransmitter dopamine at synapses. Thus, dopamine stays in the system longer, overstimulating the neuron and leading to the reward signals in the brain that lead to addiction. Using a drug to block a receptor would be of no use with cocaine addiction and would probably just make removal of dopamine even more unlikely.
47. Cocaine can be degraded by a specific enzyme that hydrolyzes an ester bond that is part of cocaine's structure. In the process of this hydrolysis, the cocaine must pass through a transition state that changes its shape. Catalytic antibodies to the transition state of the hydrolysis of cocaine hydrolyze cocaine to two harmless degradation products—benzoic acid and ecgonine methyl ester. When degraded, the cocaine cannot block dopamine reuptake. No prolongation of the neuronal stimulus occurs, and the addictive effects of the drug vanish over time.

7.8 Coenzymes

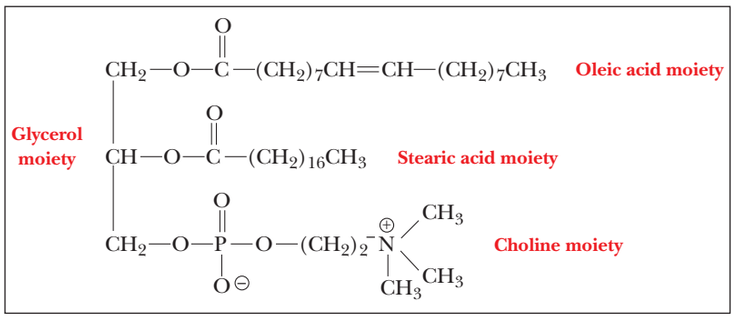
48. Nicotinamide adenine dinucleotide, oxidation–reduction; flavin adenine dinucleotide, oxidation–reduction; coenzyme A, acyl transfer; pyridoxal phosphate, transamination; biotin, carboxylation; lipoic acid, acyl transfer.
49. Most coenzymes are derivatives of compounds we call vitamins. For example, nicotinamide adenine dinucleotide is produced from the B vitamin niacin. Flavin adenine dinucleotide comes from riboflavin.
50. Vitamin B_6 is the source of pyridoxal phosphate, which is used in transamination reactions.
51. Coenzymes can accomplish the same mechanisms that the amino acids do in a reaction. For example, a metal ion may act as a general acid or base. Parts of a coenzyme, such as the reactive carbanion of thiamine pyrophosphate, may act as a nucleophile to catalyze the reaction.
52. Yes, there would be a preference. Because the coenzyme and the other substrate will be locked into the enzyme, the hydride ion would come from some functional group that had a fixed position. Therefore, the hydride would come from one side.

Chapter 8

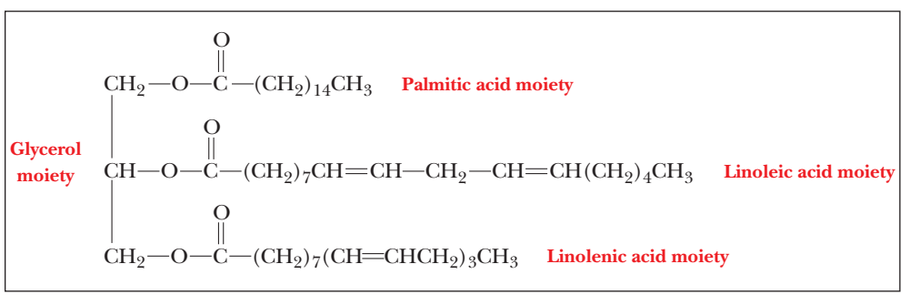
8.1 The Definition of a Lipid

1. Solubility properties (insoluble in aqueous or polar solvents, soluble in nonpolar solvents). Some lipids are not at all structurally related.

8.2 The Chemical Natures of the Lipid Types

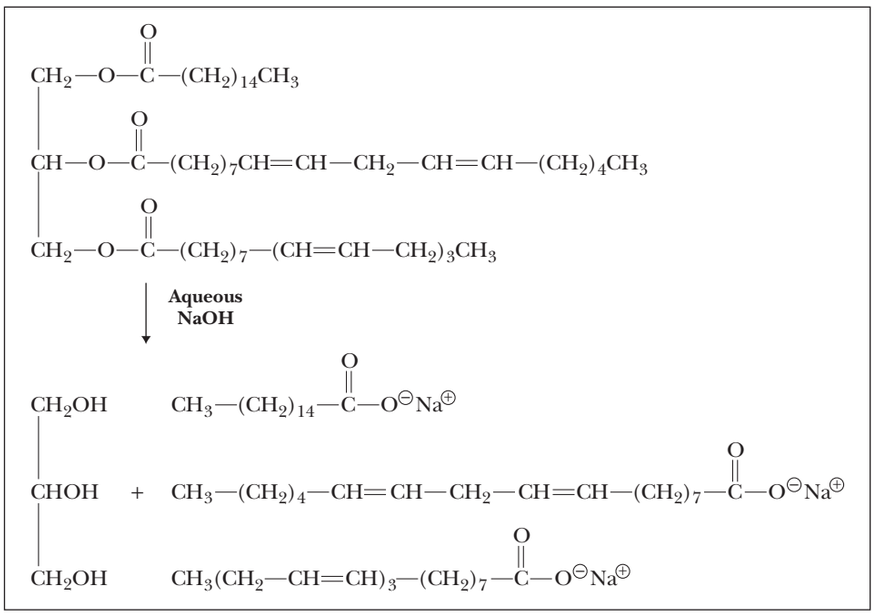
2. In both types of lipids, glycerol is esterified to carboxylic acids, with three such ester linkages formed in triacylglycerols and two in phosphatidyl ethanolamines. The structural difference comes in the nature of the third ester linkage to glycerol. In phosphatidyl ethanolamines, the third hydroxyl group of glycerol is esterified not to a carboxylic acid but to phosphoric acid. The phosphoric acid moiety is esterified in turn to ethanolamine. (See Figures 8.2 and 8.5.)
3. 

4. Both sphingomyelins and phosphatidylcholines contain phosphoric acid esterified to an amino alcohol, which must be choline in the case of a phosphatidylcholine and may be choline in the case of a sphingomyelin. They differ in the second alcohol to which phosphoric acid is esterified. In phosphatidylcholines, the second alcohol is glycerol, which has also formed ester bonds to two carboxylic acids. In sphingomyelins, the second alcohol is another amino alcohol, sphingosine, which has formed an amide bond to a fatty acid. (See Figure 8.6.)
5. This lipid is a ceramide, which is one kind of sphingolipid.
6. Sphingolipids contain amide bonds, as do proteins. Both can have hydrophobic and hydrophilic parts, and both can occur in cell membranes, but their functions are different.
7. Any combination of fatty acids is possible.



8. Steroids contain a characteristic fused-ring structure, which other lipids do not.
9. Waxes are esters of long-chain carboxylic acids and long-chain alcohols. They tend to be found as protective coatings.
10. Phospholipids are more hydrophilic than cholesterol. The phosphate group is charged, and the attached alcohol is charged or polar. These groups interact readily with water. Cholesterol has only a single polar group, an —OH.

11.



12. The waxy surface coating is a barrier that prevents loss of water.
13. The surface wax keeps produce fresh by preventing loss of water.
14. Cholesterol is not very water-soluble, but lecithin is a good natural detergent, which is actually part of lipoproteins that transport the less soluble fats through the blood.
15. The lecithin in the egg yolks serves as an emulsifying agent by forming closed vesicles. The lipids in the butter (frequently triacylglycerols) are retained in the vesicles and do not form a separate phase.
16. The removal of the oil also removes the natural oils and waxes on the feathers. These oils and waxes must regenerate before the birds can be released.

8.3 Biological Membranes

17. Triacylglycerols are not found in animal membranes.
18. Statements (c) and (d) are consistent with what is known about membranes. Covalent bonding between lipids and proteins [statement (e)] occurs in some anchoring motifs, but is not widespread otherwise. Proteins "float" in the lipid bilayers rather than being sandwiched between them [statement (a)]. Bulkier molecules tend to be found in the outer lipid layer [statement (b)].
19. The public is attuned to the idea of polyunsaturated fats as healthful. The *trans* configuration gives a more palatable consistency. Recently, however, concerns have arisen about the extent to which such products mimic saturated fats.
20. Partially hydrogenated vegetable oils have the desired consistency for many foods, such as oleomargarine and components of TV dinners.
21. Many of the double bonds have been saturated. Crisco contains "partially hydrogenated vegetable oils."
22. Less heart disease is associated with diets low in saturated fatty acids.
23. The transition temperature is lower in a lipid bilayer with mostly unsaturated fatty acids compared with one with a high percentage of saturated fatty acids. The bilayer with the unsaturated fatty acids is already more disordered than the one with a high percentage of saturated fatty acids.
24. Myelin is a multilayer sheath consisting mainly of lipids (with some proteins) that insulates the axons of nerve cells, facilitating transmission of nerve impulses.
25. At the lower temperature, the membrane would tend to be less fluid. The presence of more unsaturated fatty acids would tend to compensate by increasing the fluidity of the membrane compared to one at the same temperature with a higher proportion of saturated fatty acids.
26. The higher percentage of unsaturated fatty acids in membranes in cold climates is an aid to membrane fluidity.
27. Hydrophobic interactions among the hydrocarbon tails are the main energetic driving force in the formation of lipid bilayers.

8.4 The Kinds of Membrane Proteins

28. A glycoprotein is formed by covalent bonding between a carbohydrate and a protein, whereas a glycolipid is formed by covalent bonding between a carbohydrate and a lipid.
29. Proteins that are associated with membranes do not have to span the membrane. Some can be partially embedded in it, and some associate with the membrane by noncovalent interactions with its exterior.
30. A 100-g sample of membrane contains 50 g of protein and 50 g of phosphoglycerides.

$$50 \text{ g lipid} \times \frac{1 \text{ mol lipid}}{800 \text{ g lipid}} = 0.0625 \text{ mol lipid}$$

$$50 \text{ g protein} \times \frac{1 \text{ mol protein}}{50{,}000 \text{ g protein}} = 0.001 \text{ mol protein}$$

The molar ratio of lipid to protein is 0.0625/0.001 or 62.5/1.

31. Nature chooses what works. This is an efficient use of a large protein and of the energy of ATP.
32. In a protein that spans a membrane, the nonpolar residues are the exterior ones; they interact with the lipids of the cell membrane. The polar residues are in the interior, lining the channel through which the ions enter and leave the cell.

8.5 The Fluid-Mosaic Model of Membrane Structure

33. Statements (c) and (d) are correct. Transverse diffusion is only rarely observed [statement (b)], and the term *mosaic* refers to the pattern of distribution of proteins in the lipid bilayer [statement (e)]. Peripheral proteins are also considered part of the membrane [statement (a)].

8.6 The Functions of Membranes

34. Biological membranes are highly nonpolar environments. Charged ions tend to be excluded from such environments rather than dissolving in them, as they would have to do to pass through the membrane by simple diffusion.
35. Statements (a) and (c) are correct; statement (b) is not correct because ions and larger molecules, especially polar ones, require channel proteins.

8.7 Lipid-Soluble Vitamins and Their Functions

36. Cholesterol is a precursor of vitamin D_3; the conversion reaction involves ring opening.
37. Vitamin E is an antioxidant.
38. Isoprene units are five-carbon moieties that play a role in the structure of a number of natural products, including fat-soluble vitamins.
39. See Table 8.3.

40. The *cis–trans* isomerization of retinal in rhodopsin triggers the transmission of an impulse to the optic nerve and is the primary photochemical event in vision.
41. Vitamin D can be made in the body.
42. Lipid-soluble vitamins accumulate in fatty tissue, leading to toxic effects. Water-soluble vitamins are excreted, drastically reducing the chances of an overdose.
43. Vitamin K plays a role in the blood-clotting process. Blocking its mode of action can have an anticoagulant effect.
44. Vitamins A and E are known to scavenge free radicals, which can do oxidative damage to cells.
45. Eating carrots is good for both. Vitamin A, which is abundant in carrots, plays a role in vision. Diets that include generous amounts of vegetables are associated with a lower incidence of cancer.

8.8 Prostaglandins and Leukotrienes

46. An omega-3 fatty acid has a double bond at the third carbon from the methyl end.
47. Leukotrienes are carboxylic acids with three conjugated double bonds.
48. Prostaglandins are carboxylic acids that include a five-membered ring in their structure.
49. Prostaglandins and leukotrienes are derived from arachidonic acid. They play a role in inflammation and in allergy and asthma attacks.
50. Prostaglandins in blood platelets can inhibit their aggregation. This is one of the important physiological effects of prostaglandins.

Chapter 9

9.1 Levels of Structure in Nucleic Acids

1. (a) Double-stranded DNA is usually thought of as having secondary structure, unless we consider its supercoiling (tertiary) or association with proteins (quaternary).
   (b) tRNA is a tertiary structure with many folds and twists in three dimensions.
   (c) mRNA is usually considered a primary structure, as it has little other structure.

9.2 The Covalent Structure of Polynucleotides

2. Thymine has a methyl group attached to carbon 5; uracil does not.
3. In adenine, carbon 6 has an amino group attached; in hypoxanthine, carbon 6 is a carbonyl group.
4.

A	Adenine	Adenosine or deoxyadenosine	Adenosine-5′-triphosphate or deoxyadenosine-5′-triphosphate
G	Guanine	Guanosine or deoxyguanosine	Guanosine-5′-triphosphate or deoxyguanosine-5′-triphosphate
C	Cytosine	Cytidine or deoxycytidine	Cytidine-5′-triphosphate or deoxycytidine-5′-triphosphate
T	Thymine	Deoxythymidine	Deoxythymidine-5′-triphosphate
U	Uracil	Uridine	Uridine-5′-triphosphate

5. ATP is made from adenine, ribose, and three phosphates linked the 5′-hydroxyl of the ribose. dATP is the same, except that the sugar is deoxyribose.
6. The sequence on the opposite strand for each of the following (all read 5′ → 3′) is ACGTAT TGCATA AGATCT TCTAGA ATGGTA TACCAT.
7. They are DNA sequences because of the presence of thymine rather than uracil.
8. (a) Definitely yes! If there is anything that you don't want falling apart, it's your storehouse of genetic instructions. (Compare the effectiveness of a computer if all the *.exe files were deleted.)
   (b) In the case of messenger RNA, yes. The mRNA is the transmitter of information for protein synthesis, but it is needed only as long as a particular protein is needed. If it were long-lived, the protein would continue to be synthesized even when not needed; this would waste energy and could cause more direct detrimental effects. Thus, most mRNAs are short-lived (minutes); if more protein is needed, more mRNA is made.
9. Four different kinds of bases—adenine, cytosine, guanine, and uracil—make up the preponderant majority of the bases found in RNA, but they are not the only ones. Modified bases occur to some extent, principally in tRNA.
10. This speculation arose from the fact that ribose has three hydroxyl groups that can be esterified to phosphoric acid (at the 2′, 3′, and 5′ positions), whereas deoxyribose has free hydroxyls at the 3′ and 5′ positions alone.
11. The hydrolysis of RNA is greatly enhanced by the formation of a cyclic 2′,3′-phosphodiester intermediate. DNA, lacking the 2′-hydroxyl group, cannot form the intermediate and thus is relatively resistant to hydrolysis.

9.3 The Structure of DNA

12.

Structure	Kind of Nucleic Acid
A-form helices	Double-stranded RNA
B-form helices	DNA
Z-form helices	DNA with repeating CGCGCG sequences
Nucleosomes	Eukaryotic chromosomes
Circular DNA	Bacterial, mitochondrial, plasmid DNA

13. See Figure 9.8.
14. Statements (c) and (d) are true; statements (a) and (b) are not.
15. True. There is room for binding and access to the base pairs in both the major and minor grooves of DNA.
16. The major groove and minor groove in B-DNA have very different dimensions (width); those in A-DNA are much closer in width.
17. Statement (c) is true. Statements (a) and (b) are false. Statement (d) is true for the B form of DNA but not for the A and Z forms.
18. Supercoiling refers to twists in DNA over and above those of the double helix. Positive supercoiling refers to an extra twist in DNA caused by overwinding of the helix before sealing the ends to produce circular DNA. A topoisomerase is an enzyme that induces a single-strand break in supercoiled DNA, relaxes the supercoiling, and reseals the break. Negative supercoiling refers to unwinding of the double helix before sealing the ends to produce circular DNA.
19. Propeller-twist is a movement of the two bases in a base pair away from being in the same plane.
20. An AG/CT step is a small section of double-stranded DNA where one strand is 5′-AG-3′, and the other is 5′-CT-3′. The exact nature of such steps greatly influences the overall shape of a double helix.
21. Propeller-twist reduces the strength of the hydrogen bond but moves the hydrophobic region of the base out of the aqueous environment, thus being more entropically favorable.
22. B-DNA is a right-handed helix with specified dimensions (10 base pairs per turn, significant differences between major and minor groove, etc.). Z-DNA is a left-handed double helix with different dimensions (12 base pairs per turn, similar major and minor grooves, etc.).
23. Positive supercoils in circular DNA will be left-handed.
24. Chromatin is the complex consisting of DNA and basic proteins found in eukaryotic nuclei (see Figure 9.16).
25. See the figure in the Biochemical Connections box on triple-helical DNA (page 247).
26. Negative supercoiling, nucleosome winding, Z-form DNA.
27. It binds to the DNA, forming loops around itself. It then cuts both strands of DNA on one part of the loop, passes the ends across another loop, and reseals.
28. Histones are very basic proteins with many arginine and lysine residues. These residues have positively charged side chains under physiological pH. This is a source of attraction between the DNA and histones because the DNA has negatively charged phosphates: Histone-NH_3^+ attracts $^-$O—P—O— DNA chain.

    When the histones become acetylated, they lose their positive charge: Histone—NH—$COCH_3$. They therefore have no attraction to the phosphates on the DNA. The situation is even less favorable if they are phosphorylated because now both the histone and the DNA carry negative charges.
29. Adenine–guanine base pairs occupy more space than is available in the interior of the double helix, whereas cytosine–thymine base pairs are too small to span the distance between the sites to which complementary bases are bonded. One would not normally expect to find such base pairs in DNA.

30. The phosphate groups in DNA are negatively charged at physiological pH. If they were grouped together closely, as in the center of a long fiber, the result would be considerable electrostatic repulsion. Such a structure would be unstable.
31. The percentage of cytosine equals that of guanine, 22%. This DNA thus has a 44% G–C content, implying a 56% A–T content. The percentage of adenine equals that of thymine, so adenine and thymine are 28% each.
32. If the DNA were not double stranded, the requirement G=C and A=T would no longer exist.
33. The base distribution would not have A=T and G=C, and total purine would not be equal to total pyrimidine.
34. The purpose of the Human Genome Project was the complete sequencing of the human genome. There are many reasons for doing this. Some are tied to basic research (i.e., the desire to know all that is knowable, especially about our own species). Some are medical in nature (i.e., a better understanding of genetic diseases and how growth and development are controlled). Some are comparative in nature, looking at the similarities and differences between genomes of other species. Our DNA is at least 95% the same as that of a chimpanzee, yet we are clearly different. An understanding of our genome will help us understand what separates humankind from other primates and nonprimates.
35. Human gene therapy has many legal and ethical considerations. Some are moral and philosophical: Do we have the right to manipulate human DNA? Are we playing God? Should "tailor-made" humans be allowed? Some are more scientific: Do we have the knowledge to do it right? What happens if we make a mistake? Will a patient die that would not have died with other treatments?
36. Advantages would be that people could make informed lifestyle choices. A person with a genotype known to lead to atherosclerosis could change his or her diet and exercise habits from an early age to help fight this potential problem and could also seek preventive drug therapies. Disadvantages might involve legal issues over the right to know such information. Employers could discriminate against prospective employees based on a genotype marker that might indicate a susceptibility to drug abuse, alcoholism, or disease. A caste system based on genetics could arise.
37. Because any system involving replication of DNA by DNA polymerases must have a primer to start the reaction, the primer can be RNA or DNA, but it must bind to the template strand being read. Thus, enough of the sequence must be known to create the correct primer.

9.4 Denaturation of DNA

38. A–T base pairs have two hydrogen bonds, whereas G–C base pairs have three. It takes more energy and higher temperature to disrupt the structure of DNA rich in G–C base pairs.

9.5 The Principal Kinds of RNA and Their Structures

39. See Figures 9.20 and 9.25.
40. Small nuclear RNA (snRNA) is found in the eukaryotic nucleus and is involved in splicing reactions of other RNA types. An snRNP is a small nuclear ribonucleoprotein particle. A complex of small nuclear RNA and protein catalyzes splicing of RNA.
41. Ribosomal RNA (rRNA) is the largest. Transfer RNA (tRNA) is the smallest.
42. Messenger RNA (mRNA) has the least amount of secondary structure (hydrogen bonding).
43. The bases in a double-stranded chain are partially hidden from the light beam of a spectrophotometer by the other bases in close proximity, as though they were in the shadow of the other bases. When the strands unwind, these bases become exposed to the light and absorb it; therefore, the absorbance increases.
44. RNA interference is the process by which small RNAs prevent the expression of genes.
45. More extensive hydrogen bonding occurs in tRNA than in mRNA. The folded structure of tRNA, which determines its binding to ribosomes in the course of protein synthesis, depends on its hydrogen-bonded arrangement of atoms. The coding sequences of mRNA must be accessible to direct the order of amino acids in proteins and should not be rendered inaccessible by hydrogen bonding.
46. They prevent intramolecular hydrogen bonding (which occurs in tRNA via the usual A–U and C–G associations), thus permitting loops that are critical for function, the most important being the anticodon loop.
47. Turnover of mRNA should be rapid to ensure that the cell can respond quickly when specific proteins are needed. Ribosomal subunits, including their rRNA component, can be recycled for many rounds of protein synthesis. As a result, mRNA is degraded more rapidly than rRNA.
48. The mistake in the DNA would be more harmful because every cell division would propagate the mistake. A mistake in transcription would lead to one wrong RNA molecule that can be replaced with a correct version with the next transcription.
49. Eukaryotic mRNA is initially formed in the nucleus by transcription of DNA. The mRNA transcript is then spliced to remove introns, a poly-A tail is added at the 3′ end, and a 5′-cap is put on. This is the final mRNA, which is then transported, in most cases, out of the nucleus for translation by the ribosomes.
50. The numbers 50S, 30S, etc. refer to a relative rate of sedimentation in an ultracentrifuge and cannot be added directly. Many things besides molecular weight influence the sedimentation characteristics, such as shape and density.

Chapter 10

10.1 The Flow of Genetic Information in the Cell

1. Replication is the production of new DNA from a DNA template. Transcription is the production of RNA from a DNA template. Translation is the synthesis of proteins directed by mRNA, which reflects the base sequence of DNA.
2. False. In retroviruses, the flow of information is RNA → DNA.
3. DNA represents the permanent copy of genetic information, whereas RNA is transient. The cell could survive production of some mutant proteins, but not DNA mutation.

10.2 Replication of DNA

4. The semiconservative replication of DNA means that a newly formed DNA molecule has one new strand and one strand from the original DNA. The experimental evidence for semiconservative replication comes from density-gradient centrifugation (Figure 10.3). If replication were a conservative process, the original DNA would have two heavy strands and all newly formed DNA would have light strands.
5. A replication fork is the site of formation of new DNA. The two strands of the original DNA separate, and a new strand is formed on each original strand.
6. An origin of replication consists of a bubble in the DNA. There are two places at opposite ends where new polynucleotide chains are formed (Figure 10.4).
7. Separating the two strands of DNA requires unwinding the helix.
8. If the original Meselson–Stahl experiment had used longer pieces of DNA, the results would not have been as clear-cut. Unless the bacteria were synchronized as to their stage of development, the DNA could have represented several generations at once.
9. Replication requires separating the strands of DNA. This cannot happen unless the DNA is unwound.

10.3 DNA Polymerase

10. Most DNA-polymerase enzymes also have exonuclease activity.
11. DNA polymerase I is primarily a repair enzyme. DNA polymerase III is mainly responsible for the synthesis of new DNA. See Table 10.1.
12. The processivity of a DNA polymerase is the number of nucleotides incorporated before the enzyme dissociates from the template. The higher this number, the more efficient the replication process.
13. The reactants are deoxyribonucleotide triphosphates. They provide not only the moiety to be inserted (the deoxyribonucleotide) but also the energy to drive the reaction (dNTP → inserted NMP + PP_i, $PP_i \rightarrow 2P_i$).
14. Hydrolysis of the pyrophosphate product prevents the reversal of the reaction by removing a product.
15. One strand of newly formed DNA uses the 3′-to-5′ strand as a template. The problem arises with the 5′-to-3′ strand. Nature deals with this issue by using short stretches of this strand for a number of chunks of newly formed DNA. They are then linked by DNA ligase (Figure 10.5).
16. The free 3′ end is needed as the site to which added nucleotides bond. A number of antiviral drugs remove the 3′ end in some way.
17. The large negative ΔG° ensures that the back reaction of depolymerization does not occur. Energy overkill is a common strategy when it is critically important that the process does not go in the reverse direction.
18. Nucleophilic substitution is a common reaction mechanism, and the hydroxyl group at the 3′ end of the growing DNA strand is an example of a frequently encountered nucleophile.
19. Some enzymes have a recognition site that is not the same as the active site. In the specific case of DNA polymerase III, the sliding clamp tethers the rest of the enzyme to the template. This ensures a high degree of processivity.

10.4 Proteins Required for DNA Replication

20. All four deoxyribonucleoside triphosphates, template DNA, DNA polymerase, all four ribonucleoside triphosphates, primase, helicase, single-strand binding protein, DNA gyrase, DNA ligase.
21. DNA is synthesized from the 5′ end to the 3′ end, and the new strand is antiparallel to the template strand. One of the strands is exposed from the 5′ end to the 3′ end as a result of unwinding. Small stretches of new DNA are synthesized, still in an antiparallel direction from the 5′ end to the 3′ end and are linked by DNA ligase. See Figure 10.5.
22. DNA gyrase introduces a swivel point in advance of the replication fork. Primase synthesizes the RNA primer. DNA ligase links small, newly formed strands to produce longer ones.
23. In the replication process, the single-stranded portions of DNA are complexed to specific proteins.
24. DNA ligase seals the nicks in newly formed DNA.
25. The primer in DNA replication is a short sequence of RNA to which the growing DNA chain is bonded.
26. Specific enzymes exist to cut the DNA and give a supercoiled configuration at the replication fork that allows replication to proceed.
27. Polymerase III does not insert a deoxyribonucleotide without checking to see that the previous base is correct. It needs a previous base to check even if that base is part of a ribonucleotide.

10.5 Proofreading and Repair

28. When an incorrect nucleotide is introduced into a growing DNA chain as a result of mismatched base pairing, DNA polymerase acts as a 3′-exonuclease, removing the incorrect nucleotide. The same enzyme then incorporates the correct nucleotide.
29. In *E. coli,* two different kinds of exonuclease activity are possible for DNA polymerase I, which functions as a repair enzyme.
30. An exonuclease nicks the DNA near the site of the thymine dimers. Polymerase I acts as a nuclease and excises the incorrect nucleotides, then acts as a polymerase to incorporate the correct ones. DNA ligase seals the nick.
31. In DNA, cytosine spontaneously deaminates to uracil. The presence of the extra methyl group is a clear indication that a thymine really belongs in that position, not a cytosine that has been deaminated.
32. About 5000 books: 10^{10} characters/error × 1 book/(2×10^6 characters) = 5×10^3 books/error.
33. 1000 characters/second × 1 word/5 characters × 60 seconds/minute = 12,000 words/minute.
34. 1 second/1000 characters × 10^{10} characters/error × 107 seconds/error = 16.5 weeks/error nonstop.
35. Prokaryotes methylate their DNA soon after replication. This aids the process of mismatch repair. The enzymes that carry out the process can recognize the correct strand by its methyl groups.

    The newly formed strand, which contains the incorrect base, does not have methyl groups.
36. DNA is constantly being damaged by environmental factors and by spontaneous mutations. If these mistakes accumulate, deleterious amino acid changes or deletions can arise. As a result, essential proteins, including those that control cell division and programmed cell death, are inactive or overactive, eventually leading to cancer.
37. Prokaryotes have a last-resort mechanism for dealing with drastic DNA damage. This mechanism, called the SOS response, includes the crossing over of DNA. Replication becomes highly error-prone, but it serves the need of the cell to survive.

10.6 Eukaryotic DNA Replication

38. Eukaryotes usually have several origins of replication, whereas prokaryotes have only one.
39. The general features of DNA replication are similar in prokaryotes and eukaryotes. The main differences are that eukaryotic DNA polymerases do not have exonuclease activity. After synthesis, eukaryotic DNA is complexed with proteins; prokaryotic DNA is not.
40. Histones are proteins complexed to eukaryotic DNA. Their synthesis must take place at the same rate as DNA synthesis. The proteins and DNA must then assemble in proper fashion.
41. (a) Eukaryotic DNA replication must deal with histones; the linear DNA molecule in eukaryotes is a much larger molecule and requires special treatment at the ends.

    (b) Special polymerases are used in the organelles.
42. Eukaryotes have more DNA polymerases, which tend to be larger molecules. Eukaryotic DNA polymerases tend not to have exonuclease activity. There are more origins of replication in eukaryotes and shorter Okazaki fragments. See Table 10.5.
43. Mechanisms exist to ensure that DNA synthesis takes place only once in the eukaryotic cell cycle, during the S phase. Preparation for DNA synthesis can and does take place in the G1 phase, but the timing of actual synthesis is strictly controlled.
44. If the telomerase enzyme were inactivated, DNA synthesis would eventually stop. This enzyme maintains the 3′ template end strand so that it does not undergo degradation with each round of DNA synthesis. The degradation in turn arises from the removal of the RNA primer with each round of DNA synthesis.
45. If histone synthesis took place faster than DNA synthesis, it would be highly disadvantageous to invest the energy required for protein synthesis. The histones would have no DNA with which to bind.
46. Replication licensing factors (RLFs) are proteins that bind to eukaryotic DNA. They get their name from the fact that replication cannot proceed until they are bound. Some of the RLF proteins have been found to be cytosolic. They have access to the chromosome only when the nuclear membrane dissolves during mitosis. Until they are bound, replication cannot occur. This property links eukaryotic DNA replication and the cell cycle. Once RLFs have bound, the DNA is then competent for replication.
47. It is faster in prokaryotes. The DNA is smaller, and the lack of compartmentalization within the cell facilitates the process. DNA replication in eukaryotes is linked to the cell cycle, and prokaryotic cells proliferate more quickly than those of eukaryotes.
48. In reverse transcriptase action, the single RNA strand serves as a template for the synthesis of a single DNA strand. The DNA strand, in turn, serves as the template for synthesis of the second strand of DNA.
49. Circular DNA does not have ends. This removes the necessity for maintaining the 3′ template end on removal of the RNA primer. Telomeres and telomerase are not needed with circular DNA.
50. The presence of a DNA polymerase that operates only in mitochondria is consistent with the view that these organelles are derived from bacteria incorporated by endosymbiosis. The bacteria were originally free-living organisms earlier in evolutionary history.

Chapter 11

11.2 Transcription in Prokaryotes

1. No primer is required for transcription of DNA into RNA.
2. RNA polymerase from *E. coli* has a molecular weight of about 500,000 and four different kinds of subunits. It uses one strand of the DNA template to direct RNA synthesis. It catalyzes polymerization from the 5′ end to the 3′ end.
3. The subunit composition for the holoenzyme is $\alpha_2\beta\beta'\sigma$.
4. The core enzyme lacks the σ subunit; the holoenzyme has it.
5. The strand that the RNA polymerase uses as a template for its RNA is called the template strand, the noncoding strand, the antisense strand, and the (–) strand. The other strand, whose sequence matches the RNA produced except for the T–U change, is called the nontemplate strand, the coding strand, the sense strand, and the (+) strand.
6. The promoter region is the portion of DNA to which RNA polymerase binds at the start of transcription. This region lies upstream (nearer the 3′ end of the template DNA) of the actual gene for the RNA. The promoter regions of DNA from many organisms have sequences in common (consensus sequences). The consensus sequences frequently lie 10 base pairs and 35 base pairs upstream of the start of transcription.
7. Moving from 5′ to 3′ on the coding strand, the order is the following: Fis site, UP element, –35 region, Pribnow box, TSS.
8. Intrinsic termination of transcription involves the formation of a hairpin loop in the RNA being formed, which stalls the RNA polymerase over a region rich in A–U base pairs. This causes termination of transcription and release of the transcript. Rho-dependent termination often involves a similar hairpin loop, but, in addition, a Rho protein binds to the RNA and moves along it toward the transcription bubble. When the Rho protein reaches the transcription bubble, it causes termination.
9. See Figure 11.1. The top DNA strand is the nontemplate strand because it is not used to create the RNA. It is called the coding strand because it has the same sequence as the RNA produced, except for the change of T for U. It is called the sense strand because its sequence would give the correct

amino acid sequence of the protein product. It is called the (+) strand again because it has the correct sequence. The bottom strand is called the template strand because it is the one used to make the RNA. It is also the noncoding strand because its sequence does not match the RNA produced. It is the antisense and the (–) strand for the same reason.

11.3 Transcription Regulation in Prokaryotes

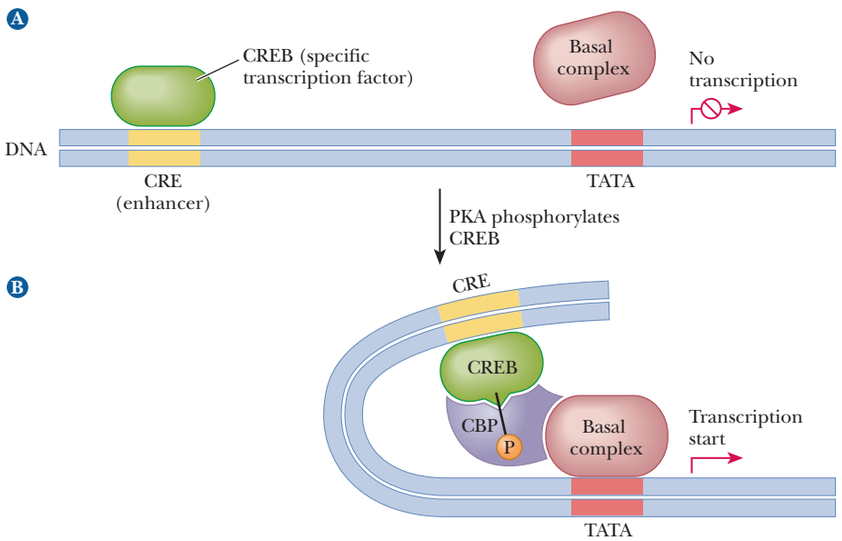
10. An inducer is a substance that leads to transcription of the structural genes in an operon. A repressor is a substance that prevents transcription of the structural genes in an operon.
11. The σ factor is a subunit of prokaryotic RNA polymerase. It directs the polymerase to specific promoters and is one of the ways that gene expression is controlled in prokaryotes.
12. σ^{70} is the normal σ-subunit for RNA polymerase in *E. coli*. It directs RNA polymerase to most of the genes that are transcribed under normal circumstances. σ^{32} is an alternate subunit that is produced when the cells are grown at higher temperatures. It directs the RNA polymerase to other genes that need to be expressed during heat shock conditions.
13. The catabolite activator protein is a transcription factor in *E. coli* that stimulates transcription of the *lac* operon structural genes. It responds to cAMP levels such that the *lac* operon is transcribed only when the cells must use lactose as a fuel source.
14. Transcription attenuation is the process found in prokaryotes in which transcription can continue or be prematurely aborted based on the concurrent translation of the mRNA produced. This is often seen in genes whose protein products lead to amino acid synthesis.
15. An operon consists of an operator gene, a promoter gene, and structural genes. When a repressor is bound to the operator, RNA polymerase cannot bind to the promoter to start transcription of the structural genes. When an inducer is present, it binds to the repressor, rendering it inactive. The inactive repressor can no longer bind to the operator. As a result, RNA polymerase can bind to the promoter, leading to the eventual transcription of the structural genes.
16. See Figure 11.5.
17. With phage SPO1, which infects the bacteria *B. subtilis,* the virus has a set of genes called the early genes that are transcribed by the host's RNA polymerase, using its regular σ-subunit. One of the viral early genes codes for a protein called gp28. This protein is another σ-subunit, which directs the RNA polymerase to preferentially transcribe more of the viral genes during the middle phase. Products of the middle phase transcription are gp33 and gp34, which together make up another σ factor that directs the transcription of the late genes.
18. See Figure 11.14. When the level of tryptophan is low, the *trp*$tRNA^{trp}$ becomes limiting. This stalls the ribosome over the tryptophan codons on the mRNA. By stalling the ribosome there, the antitermination loop can form, transcription is not aborted, and the full mRNA is produced. If the ribosome does not stall there, the termination loop forms, and the leader mRNA dissociates.

11.4 Transcription in Eukaryotes

19. Exons are the portions of DNA that are expressed, which means that they are reflected in the base sequence of the final mRNA product. Introns are the intervening sequences that do not appear in the final product, but are removed during the splicing of mRNA.
20. There are three RNA polymerases in eukaryotes, compared with one in prokaryotes. There are many more transcription factors in eukaryotes, including complexes of them necessary for polymerase recruitment. RNA is extensively processed after transcription in eukaryotes, and, in most cases, the mRNA must leave the nucleus to be translated, whereas translation and transcription can occur at the same time in prokaryotes.
21. RNA polymerase I produces most of the rRNA. RNA polymerase II produces mRNA, and RNA polymerase III produces tRNA, the 5S ribosomal subunit, and snRNA.
22. The first component includes a variety of upstream elements, which act as enhancers and silencers. Two common ones are close to the core promoter and are the GC box (–40), which has a consensus sequence of GGGCGG, and the CAAT box (extending to –110), which has a consensus sequence of GGCCAATCT. The second component, found at position –25, is the TATA box, which has a consensus sequence of TATAA(T/A). The third component includes the transcription start site at position +1 and is surrounded by a sequence called the initiator element (*Inr*). The final component is a possible downstream regulator.
23. TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH are the general transcription factors. TFIID is also the TATA-box binding protein and is associated with TAFs (TBP associated factors).
24. Its primary function is as a general transcription factor involved in the formation of the open complex for transcription initiation. It binds to the basal unit and is involved in DNA melting through a helicase activity as well as promoter clearance via phosphorylation of the CTD of RNA polymerase. In addition, it also has a cyclin-dependent kinase activity. Thus, TFIIH is involved in tying transcription and cell division together. It is also involved in DNA repair mechanisms.

11.5 Transcription Regulation in Eukaryotes

25. The heat-shock element responds to increased temperature. The metal-response element responds to the presence of heavy metals, such as cadmium, and the cyclic-AMP-response element controls a wide variety of genes based on cAMP levels in the cell.
26. CREB is a transcription factor that binds to the cAMP-response element. It is involved with the transcription of hundreds of genes based on the cAMP levels of the cell. When there is cAMP, CREB is phosphorylated, which allows it to bind the CREB binding protein, which connects the CRE to the basal transcription machinery, stimulating transcription.
27. Regulation in eukaryotes is much more complicated. Prokaryotic regulation is controlled by the choice of σ-subunit, the nature of the promoters, and the use of repressors/inducers. In eukaryotes, there are many more promoter elements, transcription factors, and coactivators. In addition, the DNA must be released from histone proteins, so transcription of DNA is linked to histone modifications.
28. As the mRNA is being produced, ribosomes are bound and begin to translate. A leader sequence on the mRNA leads to a leader peptide. Loops can form in the mRNA in different ways. Some loop combinations lead to transcription termination. The speed with which the ribosome is able to move on the mRNA controls which loop combinations form, and this speed is usually governed by the level of a specific tRNA that is available for the translation.
29. Assuming that there is a basal transcription rate for a particular gene, an enhancer would bind to a transcription factor and lead to a greater level of transcription, while a silencer would bind to a transcription factor and reduce the level of transcription below the basal rate.
30. A response element is an enhancer element that binds to a specific transcription factor and increases the level of transcription of target genes. In the case of response elements, however, this is in response to a more general cell signal, such as the presence of cAMP, glucocorticoids, or heavy metals. Response elements may control a large set of genes, and a given gene may be under the control of more than one response element.
31. As seen here, CREB binds to the CRE. When phosphorylated, it also binds to CBP and bridges to the basal transcription complex.



32. TFIID is one of the general transcription factors for RNA polymerase II. Part of it is a protein that binds to the TATA box in eukaryotic promoters. Associated in complex with the TATA box and the TBP are many proteins called TAFs, for TBP associated factors.
33. The statement is untrue. Many eukaryotic promoters do have TATA boxes, but there are also genes that lack one.
34. Transcription elongation in eukaryotes is controlled in several ways. There are pause sites at which RNA polymerase tends to hesitate. There is also antitermination at which RNA polymerase can transcribe past a normal termination point. The general transcription factor TFIIF stimulates elongation as well as initiation by helping RNA polymerase II read through pause sites. A separate elongation factor, TFIIS, is called an arrest-release factor because it stimulates RNA polymerase to resume transcription once it has hesitated at a pause site. Separate proteins also exist, called P-TEF and N-TEF, that act to positively or negatively affect elongation.
35. CREB is a ubiquitous transcription factor that has been found involved in many genes. It is phosphorylated when cAMP levels are high, which triggers the activation of the genes. CREB-mediated transcription has been implicated in cell proliferation, cell differentiation, spermatogenesis, release of somatostatin, development of mature T cells, protection of nerve cells under hypoxic conditions, circadian rhythms, adaptation to exercise, regulation of gluconeogenesis, transcription regulation of phosphoenolpyruvate carboxykinase and lactate dehydrogenase, and learning and storage in long-term memory.
36. Acidic domains, glutamine-rich domains, and proline-rich domains.

11.6 Structural Motifs in DNA-Binding Proteins

37. Helix–turn–helix motifs, zinc fingers, and basic-region leucine zippers.
38. The major DNA binding protein motifs are helix–turn–helix, zinc fingers, and basic-region leucine zippers. The helix–turn–helix motifs are organized so that the two helices of the protein fit into the major groove of the DNA. Zinc fingers are formed by combinations of cysteine and/or histidine complexed with zinc ions. A loop of protein forms around this complex, and these loops fit into the major groove of the DNA. Several such loops can be found spiraling around the DNA with the major groove. The basic-region leucine zipper has two domains. One is an area of leucines spaced out every seven amino acids. This puts them on the same side of an α-helix, which allows them to dimerize with another such protein. The basic region is high in lysine and arginine, which bind tightly to the DNA backbone via electrostatic attraction.

11.7 Posttranscriptional RNA Modification

39. Introns are spliced out. Bases are modified. A poly-A tail is put on the 3′ end of mRNA. A 5′-cap is put on mRNA.
40. One of the snURPs, U-1, is the target for destruction by the body's own immune system.
41. They both have multiple isoforms created by differential splicing of mRNA.
42. Trimming is necessary to obtain RNA transcripts of the proper size. Frequently, several tRNAs are transcribed in one long RNA molecule and must be trimmed to obtain active tRNAs.
43. Capping, polyadenylation, and splicing of coding sequences take place in the processing of eukaryotic mRNA.
44. The snRNPs are small nuclear ribonucleoprotein particles. They are the site of mRNA splicing.
45. Besides its traditional role in mRNA, tRNA, and rRNA, RNA serves other functions, such as splicing reactions, trimming reactions, and the peptide synthesis reaction of peptidyl transferase. It also has been shown that some small RNAs are produced; they act as gene silencers by binding to specific DNA sequences and blocking their transcription.
46. See Figure 11.34.
47. The Human Genome Project concluded that humans had far fewer genes than previously thought, yet we seem to be more biologically and biochemically complex. One possibility suggested to explain how so few genes could lead to so many proteins is that more proteins may be produced via differential splicing of mRNA. Thus, the same amount of DNA could lead to more gene products.

11.8 Ribozymes

48. A ribozyme is RNA that has catalytic activity without the intervention of protein at the active site. The catalytic portion of RNase P is a ribozyme. The self-splicing rRNA of *Tetrahymena* is the classic example, and it has recently been shown that the peptidyl transferase activity of the ribosome is actually a ribozyme.
49. Two mechanisms for RNA self-splicing are known. In Group I ribozymes, an external guanosine is covalently bonded at the splice site, releasing one end of the intron. The free end of the exon thus produced attacks the end of the other exon to splice the two. The intron cyclizes in the process. (See Figure 11.34.) Group II ribozymes display a lariat mechanism. The 2′-OH of an internal adenosine attacks the splice site. (See Figure 11.36.)
50. Proteins are more efficient catalysts than RNA because they have wider variations in structure and thus can tailor the active site for maximum efficiency for a given reaction.

Chapter 12

12.1 Translating the Genetic Message

1. See Figure 12.1.

12.2 The Genetic Code

2. A code in which two bases code for a single amino acid allows for only 16 (4×4) possible codons, which is not adequate to code for 20 amino acids.
3. A degenerate code is one in which more than one triplet can specify a given amino acid.
4. In the binding assay technique, various tRNA molecules, one of which is radioactively labeled with ^{14}C, are mixed with ribosomes and synthetic trinucleotides bound to a filter. If the radioactive label is detected on the filter, then it is known that the particular tRNA bound to that triplet. The binding experiments can be repeated until all the triplets are assigned.
5. The wobble base can be uracil, guanine, or hypoxanthine.
6. The codons UAA, UAG, and UGA are the stop signals. These codons are not recognized by any tRNAs, but they are recognized by proteins called release factors. A release factor not only blocks the binding of a new aminoacyl-tRNA but also affects the activity of the peptidyl transferase, so that the bond between the carboxyl end of the peptide and the tRNA is hydrolyzed.
7. Note that the sequence in the codon of mRNA is reversed because mRNA synthesis is antiparallel.
   (a) Position 1 has an intermediate effect. For purine changes, a different amino acid results in all cases. The changes tend to be conservative, with only four of the 16 possible changes leading to hydrophobic-hydrophilic differences. For our purposes, glycine is considered neither hydrophobic nor hydrophilic. The resulting protein would have a better chance of functioning than in a second-base change, but a lesser probability than in a third-base change.
   (b) Position 2 is the most informational: a different amino acid results from any change. In this case, however, the chances are high (75%) that the mutation would be a conservative one, with one hydrophobic amino acid replacing another one, so the protein would still have a good chance of functioning. A change involving serine or threonine (25% chance) would alter the polarity but would not introduce a charge on the side chain; the protein might still function.
   (c) There is a high probability of a change in the type of amino acid, including differences in charge; the probability of the resulting protein having proper function is considerably lower.
   (d) Position 3 is the least informational. There is a high probability of getting the same amino acid. The protein thus has a very good chance of functioning.
8. The concept of wobble specifies that the first two bases of a codon remain the same, while there is room for variation in the third base. This is precisely what is observed experimentally.
9. Hypoxanthine is the most versatile of the wobble bases; it can base pair with adenine, cytosine, or uracil.
10. It is quite reasonable. When codons for a given amino acid have one or two nucleotides in common, a mutation is less likely to give rise to a nonfunctional protein. The survival value of such a feature guarantees its selection in evolution.
11. An ambiguous code would allow for variation in the amino acid sequence of proteins. Consequently, there would be variation in function, including a number of nonfunctional proteins.
12. Variations in the genetic code in mitochondria support the idea of their existence as free-living bacteria early in evolutionary history.

12.3 Amino Acid Activation

13. The hydrolysis of ATP to AMP and PP_i provides the energy to drive the activation step.

14. Proofreading in amino acid activation takes place in two stages. The first requires a hydrolytic site on the aminoacyl-tRNA synthetase; incorrect amino acids that have become esterified to the tRNA are removed here. The second stage of proofreading requires the recognition site on the aminoacyl-tRNA synthetase for the tRNA itself. The incorrect tRNA does not bind tightly to the enzyme.
15. The following factors ensure fidelity in protein synthesis. Aminoacyl-tRNA formation includes a high degree of enzyme specificity to connect the right amino acid to the right tRNA, proofreading in the formation of some aminoacyl-adenylates, and energy "overkill." Other factors include proper hydrogen bonding of mRNA to the ribosome and between codon and anticodon. (The latter is a relatively slow association, allowing time for mismatches to dissociate before the peptide bond is formed.) The fidelity of protein synthesis is low compared with DNA synthesis, which has proofreading procedures in addition to energy overkill and proper base pairing. The fidelity of protein synthesis is relatively high compared with RNA synthesis, which has only energy overkill and proper base pairing.
16. A separate synthetase exists for each amino acid, and this synthetase functions for all of the different tRNA molecules for that amino acid.
17. The linkage of amino acids to tRNA is as an aminoacyl ester.
18. Proofreading at the activation step allows for selection of both the amino acid and the tRNA. If proofreading took place at the level of codon–anticodon recognition, there would not be a mechanism to ensure that the correct amino acid has been esterified to the tRNA.
19. The overall process of amino acid activation is energetically favored because of the energy contributed by the hydrolysis of two phosphate bonds. Without that input of energy, it would not be favorable.

12.4 Prokaryotic Translation

20. Peptidyl transferase catalyzes the formation of a new peptide bond in protein synthesis. The elongation factors, EF-Tu and EF-Ts, are required for binding of aminoacyl tRNA to the A site. The third elongation factor, EF-G, is needed for the translocation step in which the mRNA moves with respect to the ribosome, exposing the codon for the next amino acid.
21. The initiation complex in *E. coli* requires mRNA, the 30S ribosomal subunit, fmet-tRNA^{fmet}, GTP, and three protein-initiation factors, called IF-1, IF-2, and IF-3. The IF-3 protein is needed for the binding of mRNA to the ribosomal subunit. The other two protein factors are required for the binding of fmet-tRNA^{fmet} to the mRNA-30S complex.
22. Attachment of the 50S ribosomal subunit to the 30S subunit in the initiation complex is needed for protein synthesis to proceed to the elongation phase.
23. The A site and the P site on the ribosome are both binding sites for charged tRNAs taking part in protein synthesis. The P (peptidyl) site binds a tRNA to which the growing polypeptide chain is bonded. The A (aminoacyl) site binds to an aminoacyl tRNA. The amino acid moiety is the next one added to the nascent protein. The E (exit) site binds the uncharged tRNA until it is released from the ribosome.
24. Puromycin terminates the growing polypeptide chain by forming a peptide bond with its C-terminus, which prevents the formation of new peptide bonds (see Figure 12.14).
25. The stop codons bind to release factors, proteins that block binding of aminoacyl tRNAs to the ribosome, and to release the newly formed protein.
26. In the course of protein synthesis, mRNA binds to the smaller ribosomal subunit.
27. The Shine–Dalgarno sequence is a purine-rich leader segment of prokaryotic mRNA. It binds to a pyrimidine-rich sequence on the 16S rRNA part of the 30S ribosomal subunit and aligns it for proper translation, beginning with the AUG start codon.
28. Your friend is mistaken. The hydrogen-bonded regions contribute to the overall shape of the tRNA. Hydrogen-bonded regions are also important in the recognition of tRNAs by aminoacyl-tRNA synthetases.
29. Methionine bound to tRNA^{fmet} can be formylated, but methionine bonded to tRNA^{met} cannot be.
30. Different tRNAs and different factors are involved. Initiation requires IF-2, which recognizes fmet-tRNA^{fmet} but not met-tRNA^{fmet}. Conversely, in elongation, EF-Tu recognizes metRNA^{met} but not fmet-tRNA^{fmet}.
31. The methionine anticodon (UAC) on the tRNA base pairs with the methionine codon AUG in the mRNA sequence that signals the start of protein synthesis.
32. The fidelity of protein synthesis is assured twice during protein synthesis—the first time during activation of the amino acids and the second time during the matching of the codon to the anticodon on the mRNA.
33. (a) Activation cycles needed for a protein with 150 AA: 150.
    (b) Initiation cycles needed for a protein with 150 AA: 1.
    (c) Elongation cycles needed for a protein with 150 AA: 149.
    (d) Termination cycles needed for a protein with 150 AA: 1.
34. Four high-energy phosphate bonds per amino acid: two in aminoacyl-tRNA formation, one in elongation with EF-Tu, and one in translocation from the A to the P site, involving EF-G. Forming a peptide bond requires about 5 kcal/mol. This is an expenditure of about 30 kcal/mol peptide bonds. This is the price of low entropy and high fidelity.
35. Not very precisely. Ignoring any editing or proofreading costs, a maximum value can be calculated in terms of high-energy phosphate bonds. We will designate each phosphate bond as ~P. Four are needed per amino acid, and two are needed per ribonucleotide or deoxyribonucleotide. Therefore, four ~P per amino acid × six ~P per codon × six ~P per DNA triplet = 144 ~P per amino acid (approximately 1050 kcal per mole of amino acid). However, the actual value would be much less because of several factors. A single mRNA molecule can be involved in the synthesis of several to many protein molecules before it is degraded. One gene can be involved in the synthesis of many mRNA molecules, with replication taking place only once per cell generation. In addition, rRNA and tRNA are relatively long-lived and available for repeated protein syntheses.
36. The fact that peptidyl transferase is one of the most conserved sequences in all of biology may indicate that it evolved very early in evolution and that it is so critical for all living organisms that it cannot be modified.
37. The less highly purified ribosome preparations contained polysomes, which are more active in protein synthesis than single ribosomes.
38. At first, peptide-bond formation was catalyzed by RNA. In time, as protein catalysts developed and became more efficient, proteins became an integral part of the ribosome.
39. Electron microscopy can give information about ribosomal structure and function, but X-ray crystallography has given far more detailed information.
40. Because the tRNAs are bound in proximity to each other on the ribosome, the growing polypeptide chain and the amino acid to be added are also close to each other. This facilitates formation of the next peptide bond.
41. A virus takes over the protein-synthesizing machinery of the cell. It uses its own nucleic acids and the cell's ribosomes.

12.5 Eukaryotic Translation

42. Similarities between protein synthesis in bacteria and protein synthesis in eukaryotes: same start and stop codons; same genetic code; same chemical mechanisms of synthesis; interchangeable tRNAs. Major differences: in prokaryotes, the Shine–Dalgarno sequence and no introns; in eukaryotes, the 5′-cap and 3′-tail on mRNA and introns have been spliced out.
43. The original N-terminal methionine can be removed by posttranslational modification.
44. Puromycin would be useful for treatment of a viral infection, but chloramphenicol would not. Viral mRNAs are translated by eukaryotic translation systems, so one must use an antibiotic active on eukaryotic systems.
45. Protein synthesis in prokaryotes takes place as a coupled process with simultaneous transcription of mRNA and translation of the message in protein synthesis. This is possible because of the lack of compartmentalization in prokaryotic cells. In eukaryotes, mRNA is transcribed and processed in the nucleus and only then exported to the cytoplasm to direct protein synthesis.
46. Some mutations can introduce stop codons. It is useful to a cell to have some mechanism to suppress the formation of incomplete proteins.

12.6 Posttranslational Modification of Proteins

47. Hydroxyproline is formed from proline, an amino acid for which there are four codons, by posttranslational modification of the collagen precursor.

12.7 Protein Degradation

48. Ubiquitin is a small polypeptide (76 amino acids) that is highly conserved in eukaryotes. When ubiquitin is linked to a protein, it marks that protein for degradation in a proteasome.
49. If proteins to be degraded did not have some signal marking them, the process would take place more randomly and thus be less efficient.
50. If protein degradation took place at any location in a cell, indiscriminate breakdown of functional proteins could take place, so this is an unlikely occurrence. It is much more useful to the cell to have a mechanism for tagging proteins to be degraded and to do so at a specific location in the cell.

Chapter 13

13.1 Purification and Detection of Nucleic Acids

1. Safety, no need for special licensing, and convenience of disposal.
2. DNA is labeled with ^{32}P and run on a gel. The gel is placed next to X-ray paper, which is then developed. The radioactivity shows up as black bands on the X-ray paper. This is called an autoradiograph.
3. The DNA run on electrophoresis gels is usually cleaved with restriction enzymes to give linear pieces; thus the shape is uniform for DNA. The charge is a constant for DNA in that every nucleotide has the same charge due to the phosphate groups; thus, DNA has a uniform shape and a uniform charge-to-mass ratio, so it separates solely on size, with the shorter fragments traveling fastest through the gel.

13.2 Restriction Endonucleases

4. The use of restriction endonucleases with different specificities gives overlapping sequences that can be combined to give an overall sequence.
5. Restriction endonucleases do not hydrolyze a methylated restriction site.
6. The restriction site of the DNA of the organism that produces a restriction endonuclease is modified, usually by methylation.
7. The restriction fragments of different sizes (restriction-fragment length polymorphisms, or RFLPs) that come about as a result of different base sequences on paired chromosomes were used as genetic markers to determine the exact position of the cystic fibrosis gene on chromosome 7.
8. An endonuclease is an enzyme that cuts nucleic acid chains in the middle, as opposed to cleaving from the ends inward. The term *restriction* came from the restricted growth seen in host cells that are infected by bacteriophages when the bacteria have restriction enzymes that can cleave the viral DNA.
9. They are all palindromes (ignoring punctuation and spacing in the latter two cases), analogous to palindromic sequences of bases in DNA. Just as the five examples are distinguished by being pronounced differently, different palindromes in DNA are distinguished and acted on by different, very specific restriction endonucleases.
10. GGATCC, GAATTC, AAGCTT (remember that these are listed 5′ to 3′, so you must read the complementary strand 5′ to 3′ to see that the sequence is the same).
11. *Hae*III cuts at a sequence of four bases, cuts in the middle of the sequence, and leaves blunt ends. *Bam*HI cuts at a sequence of six bases, cuts on the second base from the 5′ end, and leaves sticky ends.
12. Sticky ends are short regions of single-stranded DNA extending from the ends of double-stranded DNA molecules. These are produced by some restriction enzymes or can be added chemically to blunt-ended double-stranded DNA. They are important because they provide a means for DNA from different sources (e.g., "foreign" gene and plasmid, both containing sticky ends) to find each other by hydrogen bonding between complementary bases. A ligase is then used to covalently link the two molecules.
13. An advantage of using *Hae*III is that it yields blunt ends. Thus, one could combine DNA cut with this enzyme with any other DNA that also had blunt ends. Enzymes exist that quickly remove the sticky overhangs from other restriction enzymes. The disadvantage is that *Hae*III is specific for a four-base sequence that is likely to occur many times in a genome, so the target DNA may also be cleaved somewhere in the middle. Also, the blunt ends make it more difficult to get specific ligation of two DNA types.

13.3 Cloning

14. A portion of exogenous DNA is introduced into a suitable vector, frequently a bacterial plasmid, and many copies of the DNA are produced when the bacteria grow. Viruses are also commonly used as vectors.
15. The most common vectors are bacterial plasmids. Viruses and cosmids can also be used, depending on the size of the foreign DNA that must be inserted.
16. The plasmid to be used as a vector needs markers both for uptake of the target DNA sequence into the plasmid and for insertion of the plasmid into host cells. Typically, a plasmid has a gene for ampicillin resistance. Only cells that have taken up a plasmid can grow on ampicillin plates. The foreign DNA is usually inserted into a second marker to select for those plasmids that took up the target DNA. This second marker may be another antibiotic-resistant gene or some other gene, such as the β-galactosidase gene.
17. The key feature of a plasmid capable of blue/white screening is the gene for the α-subunit of the enzyme β-galactosidase. These plasmids are used with a strain of *E. coli* that are deficient in the α-subunit of this enzyme. β-Galactosidase can convert a colorless sugar derivative, called X-gal, to a blue one. The site for cleavage of the plasmid by a restriction endonuclease lies within the β-galactosidase gene. Cells that have acquired a plasmid can grow on ampicillin. If the plasmid reclosed on itself without the target DNA, the colonies that took up that plasmid grow blue. Cells that have acquired the DNA insert cannot produce a blue color.
18. Restriction enzymes to cut DNA, DNA ligase to rejoin DNA, a suitable vector to carry the foreign DNA, a cell line to accept the vector, and a way of selecting for the correct transformants.
19. Since most recombinant DNA occurs with bacterial and viral vectors, a big concern is that a mutated virus or bacteria will be released that can infect other species and that may be resistant to drugs, thereby creating a new, potentially lethal disease. Precautions include frequent sterilization of cultures to make sure that they are all dead before disposal, working in laminar hoods that isolate the recombinant DNA from the outside, and care in the choice of vectors. Some vectors that are replication-deficient outside certain cell types are used so that they cannot replicate outside the lab environment.

13.4 Genetic Engineering

20. To increase disease resistance, resistance to pests, shelf life, level of nitrogen fixation (protein content), and resistance to temperature extremes.
21. Insulin, human growth hormone, tissue plasminogen activator, enterokinase, erythropoietin, and interferon.
22. The corn being grown in the field has been genetically engineered. The gene that was introduced came from the bacterium *Bacillus thuringensis*.
23. LDH 3 has the subunit composition H_2M_2. Each of the subunits is coded for by a separate gene, so in order to clone LDH 3, one would have to clone the gene for the M subunit and the gene for the H subunit. These would be separate cloning experiments. Each gene would be cloned into an expression cell line, and the proteins would be expressed. The individual subunits could then be combined, and they would form tetramers, some of which would be LDH 3. This could be verified by native gel electrophoresis.
24. An expression vector, such as pET 5 plasmid, has the components of any normal cloning vector (e.g., origin of replication, selectable marker, multiple cloning site), but it also has the ability to have the inserted DNA be transcribed. It has a promoter for RNA polymerase, such as T7 polymerase, and a termination sequence. These border the multiple cloning site. These vectors are used with a cell line that makes T7 RNA polymerase when induced.
25. A fusion protein is a combination of a protein coded for by an expression vector and the target gene. A common one is a histidine tag and enterokinase, which are linked to the target protein when transcribed and translated. They are used to help with the eventual purification of the target protein. The overexpressed target protein can be quickly separated from the rest of the host's proteins by purifying the fusion protein, which has characteristics that make it easy to purify.
26. The bovine growth hormone is a protein that is denatured and digested in the intestinal tract. Also, all cow's milk contains some of the hormone.
27. The DNA sequence to be inserted in the bacterial plasmid to direct the production of α-globin should be cDNA, which is a sequence complementary to the mRNA for α-globin. The cDNA can be produced on the mRNA template in a reaction catalyzed by reverse transcriptase.
28. Isolate the DNA that codes for the growth factor by means of suitable probes. Introduce the DNA into a bacterial genome. Allow the bacteria to grow and to produce human growth hormone.
29. The public is concerned about contamination with prions, which come from mammalian sources. If a mammalian protein can be expressed in large quantities in bacteria, there will be no risk of prion contamination.

13.5 DNA Libraries

30. A DNA library is a collection of cells that carry cloned pieces of the entire DNA genome of an organism. A cDNA library is made by taking the mRNA from an organism, converting it to cDNA, and cloning that for the library. In this way, the active DNA sequence is stored.
31. If a DNA library is to represent the total genome of an organism, it must contain at least one clone for each DNA sequence. This requires several hundred thousand separate clones to ensure that every sequence is represented.
32. The amount of work involved in constructing a DNA library makes it desirable to have such libraries available to the entire scientific community, thus avoiding duplication of effort.

13.6 The Polymerase Chain Reaction

33. The polymerase chain reaction depends on repeated cycles of separation of DNA strands followed by annealing of primers. The first step requires a significantly higher temperature than the second, giving rise to the requirement for strict temperature control.

34. Part of the procedure of the polymerase chain reaction requires the use of high temperatures. When a temperature-stable RNA polymerase is used, there is no need to add fresh batches of enzyme for each round of amplification. This would need to be the case, however, if the RNA polymerase could not withstand the high temperatures.
35. Good primers have similar G–C contents for the forward and reverse reactions, have minimal secondary structure possibilities with each other or with themselves, and are long enough to give sufficient specificity for the gene to be duplicated without costing too much.
36. The contaminating DNA as well as the desired DNA is amplified at each stage of the polymerase chain reaction, giving rise to an impure product.
37. (a) The primers have very different G–C contents.
    (b) The forward primer will have significant secondary structure with itself (hairpin loop due to inverted Gs and Cs on end).
    (c) The forward and reverse primers will bind to each other.

13.7 DNA Fingerprinting

38. The polymerase chain reaction can increase the amount of a desired DNA sample by a considerable factor, making possible definite identification of DNA samples that were too small to be characterized by other means. It can be used on hair and blood samples found at the scene of a crime to establish the presence of a suspect. This method can also be used to identify remains of possible murder victims.
39. It is easier to show that two DNA samples do not match than to prove that they are identical.

13.8 Sequencing DNA

40. 5′GATGCCTACG3′
41. Two factors are involved here. First, large polymers must be cleaved into smaller, manageable fragments for sequencing. Enzymes (endoproteases) that cleave proteins, while showing some specificity, are far from absolutely specific, and messy mixtures result. On the other hand, restriction endonucleases are absolutely specific for palindromic base sequences in DNA, and "clean" cuts result, allowing easier purification. (Note that if the gene for a protein isn't available but the mRNA is, the resulting cDNA can be made using reverse transcriptase.) A second factor is that only relatively short fragments of protein can be sequenced without additional internal cleavage. For example, the Edman degradation is limited to peptides of about 50 amino acids or fewer. With DNA, the dideoxy method coupled with polyacrylamide-gel separation can handle DNA fragments 10 to 20 times longer.
42. DNA often has introns in the gene, so knowing the DNA sequence may give the wrong answer for the final protein sequence. Also, proteins are modified posttranslationally, so there may be modifications to the protein sequence not reflected in the DNA.
43. Open-ended answer.
44. *Benefits:* A person at risk for future heart disease could be more careful with diet and exercise. Such a person might also take a drug beforehand that would help prevent the condition from developing. Doctors with access to such information would be able to make better diagnoses and to suggest quicker treatments.

    Detriments: Employment could be based on a preconceived idea of what a good genotype is. Health and life insurance could be denied to people considered to have a risky genotype. A new type of prejudice against the "genotypically challenged" could arise.

13.9 Genomics and Proteomics

45. The genome is the total DNA of a cell, containing all the genes of that organism. The proteome is the total complement of proteins.
46. A proteomic analysis has been done on the fruit fly *Drosophila melanogaster*.
47. Using robotic technology, a slide or "chip" is loaded with thousands of specific single-stranded DNA sequences. RNA is collected from samples to be tested and converted to cDNA carrying a fluorescent tag. The sample is placed over the chip and the cDNA allowed to bind. A fluorometer measures the fluorescence from the chip and indicates which DNA sequences were bound with their corresponding cDNA. This tells researchers which genes were active as only the active genes would produce RNA.
48. Yeast could be grown under the two conditions and the mRNA collected. The mRNA could then be converted to cDNA and each population could be labeled with a different color fluorescent marker. These samples could then be overlaid on a gene chip containing the yeast genome. The color of the fluorescence on the gene chip would then tell which genes were active under the two conditions.
49. Cancerous cells have altered metabolism at the genetic level. The gene expression patterns seen in patients with known types of cancer act like a fingerprint of that type of cancer. Tissue samples from patients to be diagnosed can be used to collect the RNA and convert it to cDNA. These cDNA samples are then overlaid on a gene chip of the human genome and the binding pattern analyzed through fluorescence. The pattern seen can then be compared to the patterns seen in the known cancers to aid in the diagnosis.
50. DNA microarrays have thousands of bound single-stranded DNA spots. They are used to test for the presence of the corresponding mRNA in a biological sample via cDNA produced from the mRNA. Protein arrays, on the other hand, have applied samples of very specific and pure antibodies. Biological tissue samples are placed on the protein chip. If the antigens for the specific antibodies are present, they bind to the antibodies. Another set of antibodies with fluorescent labels is then added, and the chip analyzed with a fluorometer. The patterns seen show which antigens the tissue sample had, which can be used to diagnose the patient.

Chapter 14

14.1 Viruses

1. Some viruses have DNA and some have RNA. In some cases, a viral genome is single-stranded and in others it is double-stranded.
2. (a) The virion is the entire virus particle.
   (b) The capsid is the protein coat that surrounds the viral nucleic acid.
   (c) The nucleocapsid is the combination of the nucleic acid and the capsid.
   (d) A protein spike is a membrane-bound protein that is used to help the virus attach to its host.
3. The main factors determining the family of a virus is whether its genome is DNA or RNA and whether it has a membrane envelope. Whether the nucleic acid is single- or double-stranded and the method of incorporation of the virus are also considered.
4. The virus attaches to a specific protein on the host cell's membrane and injects its nucleic acid inside the cell.
5. In the lytic pathway, the viral nucleic acid is replicated in the host cell and packaged into new virus particles that lyse the host cell. In the lysogenic pathway, the viral DNA is incorporated into the host DNA.
6. There is no correlation. Some viruses, such as Ebola virus, are fast acting and very lethal; others, such as HIV, are slow and just as lethal. The influenza virus is fast-acting, but it is rarely lethal these days.
7. One good choice would be a drug that attacks one of the specific protein spikes on the virus. This may be an antibody that attacks it, or a drug that blocks its ability to attach to the host cell. Another choice would be a drug that inhibits a key viral enzyme, such as the reverse transcriptase of a retrovirus, or the enzymes involved in repackaging the viruses.
8. Viruses can often switch from one pathway to another, based on the condition of the host cells. If the host is healthy, there is sufficient material to allow the virus to replicate and to produce new virions. If the host cell is starved or unhealthy, there may be insufficient energy and material to do so. In this case, lysogeny allows the DNA to incorporate in the host cell, where it can wait until the cell's health improves.
9. One example would be someone who had helper T-cells lacking a CD4 receptor. The HIV virus must bind to the CD4 receptor as part of its attachment process.

14.2 Retroviruses

10. A retrovirus has an RNA genome that must pass through a stage in which it is reverse-transcribed to DNA, and this DNA must recombine with the host's DNA.
11. Reverse transcriptase.
12. The first is that retroviruses have been linked to cancer. The second is that human immunodeficiency virus (HIV) is a retrovirus. The third is that retroviruses can be used in gene therapy.
13. Gene therapy is the process of introducing a gene into the cells of an organism that was missing functional copies of the gene.
14. Ex vivo gene therapy, in which the cells are removed from the patient before being infected with the virus carrying the therapeutic gene, and in vivo gene therapy, in which the patient is directly infected with the virus carrying the gene.

15. The two most common are the Maloney murine leukemia virus (MMLV) and adenovirus. Both must be manipulated so that the critical genes for replication are removed and replaced with an expression cassette containing the therapeutic gene.
16. When retroviruses, such as MMLV, are used, there is the danger that the therapeutic gene will incorporate in a place that will disrupt another gene. In more cases than would be predicted by random chance, this seems to occur in a place that disrupts a tumor-suppressor gene, causing cancer. There is also the danger that the patient will have a strong reaction to the virus used to introduce the therapeutic gene. In at least one case, this has had fatal consequences.
17. The biggest consideration is where the therapeutic gene has to go. Some viruses are very specific to their target cells, so if the problem is in the lungs, then a virus that is good at attacking lung cells, such as adenovirus, is a good choice. In this case, in vivo delivery would be superior, because lung cells cannot be removed from the body and then replaced. However, if the problem is in an immune cell, then bone marrow cells can be removed and transformed and later given back to the patient, making ex vivo delivery an option.
18. There are dangers inherent to all forms of gene therapy. People who have SCID have such compromised immune systems that they cannot lead normal lives, and few other remedies allow them to lead normal lives. That made SCID a prime candidate for experimental techniques. Diabetes can be controlled effectively by other techniques that are well established and not as risky.

14.3 The Immune System

19. AIDS is the most well-known problem of a malfunctioning immune system. SCID is also high on the list. All allergies are immune system problems, as are autoimmune diseases. Many forms of diabetes are caused by an autoimmune disease in which a person's pancreatic cells are attacked by the immune system.
20. Innate immunity refers to a variety of protective processes, including skin, mucus, and tears as a first line of defense, and dendritic cells, phagocytes, macrophages, and natural killer cells as a second line of defense. These are always present, and the innate-immunity cells are always circulating in the body. Acquired immunity refers to the processes involving B cells and T cells, in which specific sets of them are activated in response to an antigen challenge, and these subsets then multiply.
21. One part includes physical barriers, such as skin, mucus, and tears. The cells of the innate immune system are dendritic cells, macrophages, and natural killer (NK) cells.
22. B cells, which make antibodies, killer T cells, which attack infected cells, and helper T cells, which help activate B cells.
23. MHCs are receptors on antigen-presenting cells. They bind to fragments of antigens that have been degraded by the infected cell and display it on their surface. T cells then bind to the infected cells.
24. Clonal selection refers to the process in which a particular T cell or B cell is stimulated to divide. Only the one bearing the correct receptor for the antigens being presented is selected.
25. The cells of the innate system initially attack a pathogen, such as a virus, bacteria, or even a cancerous cell. They then present antigens from the pathogen on their surfaces via their MHC proteins. The acquired immunity cells then recognize the MHC/antigen complex, bind to it, and begin the involvement of the acquired immunity system.
26. Interferon is a cytokine produced in very small quantities that stimulates natural killer cells, which attack cancerous cells. One of the first treatments for cancer was to give the patient interferon to stimulate NK cells. Having a large supply of cloned interferon is helpful, therefore, in fighting cancer.
27. When T cells and B cells are developing, they are, in a sense, "trained." If they contain receptors that recognize self-antigens, they are eliminated when they are still young. If they don't ever see any antigens they recognize, then they die by neglect. This leaves a set of precursors to T cells and B cells with receptors that recognize foreign antigens but not self-antigens.
28. Macrophages, part of the innate immune system, are the "double-edged sword." Their presence is important to attack cancer cells, and if they do a thorough job, then the cancer cells are all destroyed. However, they also cause inflammation, which has recently been shown to indirectly lead to the progression of the cancer cells that survive.
29. The small noncoding RNA (ncRNA) of the herpes virus has been linked with its ability to evade the immune system.
30. The herpes virus produces a ncRNA that stabilizes the respiratory chain of the mitochondria of the host cell. This prevents the early destruction of the infected cell by the host's immune system. At the same time, a micro RNA (miRNA) produced by the virus inhibits production of a protein on the surface of the cell that would otherwise attract NK cells.

14.4 Cancer

31. Cancer cells continue to grow and divide in situations in which normal cells do not, such as when they are not receiving growth signals from surrounding cells. They also continue to grow even if surrounding tissues are sending out "stop growth" signals. Cancer cells can co-opt the body's vascular system, causing the growth of new blood vessels to supply the cancerous cells with nutrients. Cancer cells are essentially immortal. They can continue to grow and to divide indefinitely. Cancer cells can break loose, travel to other parts of the body, and create new cancerous areas, a process known as metastasis.
32. A tumor suppressor is a molecule that restricts the ability of a cell to grow and to divide. An oncogene is a gene whose product stimulates a cell to grow and to divide.
33. The protein called p53 is a tumor suppressor. Mutations of p53 have been found in more than half of all human cancers. Ras is involved in cell division, and mutations in this protein are involved in 30% of human tumors.
34. Viruses have been implicated in many cancers. Retroviruses are particularly dangerous because they insert their DNA into the host's DNA. When this happens in a tumor-suppressor gene, the tumor suppressor is inactivated, causing cancer. Also, the homology between proto-oncogenes and oncogenes makes it likely that the infection cycle of viruses may be responsible for some proto-oncogenes becoming oncogenic.
35. Virotherapy is the process of using a virus to attempt to treat cancer. There are two strategies for virotherapy. One is to use the virus to attack and kill cancer cells directly. In this case, the virus has a protein on its surface that is specific for a cancer cell. Once inside, it kills the cancer cell. The second is to have the virus ferry a gene into the cancer cell that makes the cell more susceptible to a chemotherapeutic agent.
36. If smoking caused cancer, then everyone who smokes would have cancer, but this is not true. Smoking has been linked to cancer, and it is a strong predictor of future cancer, but cancer is the result of many things going wrong in a cell, and there is no single, definitive cause.
37. A tumor suppressor is a protein that helps control cell growth and division. It is like the brakes on a car, trying to slow down a process. Many cancers are related to mutation of tumor suppressors. An oncogene produces something that stimulates growth and division. This is like the accelerator of the car. Many other cancers are caused ultimately by overactivation of an oncogene.
38. Ras, Jun, and Fos are all considered oncogenes. In the process of cell division, Ras is a necessary component, but it is usually active only when the cell should be dividing. Oncogenic forms of Ras are overactive and lead to too much cell division. Ras is an early step in the process. Jun and Fos are transcription factors that together make up AP-1, which is involved in the transcription activation pathway involving CBP.
39. Many of the early trials involved specific delivery of an active p53 gene via gene therapy. However, such delivery is impractical for human patients in many cases. Now researchers are looking for drugs that can be taken that will increase the levels of p53.
40. Two drugs, Prima-1 and CP-31398 reactivate mutant p53; nutlins inhibit a protein called MDM2, which is itself a natural inhibitor of p53.
41. p53 can be restored in several ways. One way would be through gene therapy to give the patient functioning copies of the p53 gene if he or she lacks it. Another is to neutralize molecules that naturally inhibit p53. Another is to give the patient drugs that stimulate the production of p53 by stimulating the transcription of the p53 gene. Finally, one could use drugs that would inhibit the transcription of molecules that act as inhibitors of p53.
42. The innate immune system is instrumental in fighting cancer cells. Cell that turn cancerous display specific molecules on their surfaces that act as a help signal. Cells of the innate immune system such as macrophages and natural killer cells attack cells that display these cancer-linked antigens on their surfaces. Often they destroy the cancerous cell, ending the threat. However, if they do not, the presence of the innate immune cell can lead to inflammation. More and more research is showing that inflammation is the switch that takes a precancerous cell and turns it into a full-fledged cancer cell. Thus, innate immune cells that attack a cancer cell but fail to kill it may just make it stronger.
43. The realization that cancer's progression is fueled by inflammation has led to a theory by some scientists that we should spend more time focusing on the symptoms rather than the cure. They believe it is possible that even though potential cancer cells exist, they may not ever grow and spread if we could stop the inflammation.

Chapter 15

15.1 Standard States for Free-Energy Changes

1. There is a connection, and it is one of the most important points in this chapter. It can be expressed in the equation $\Delta G^{\circ\prime} = -RT \ln K_{eq}$.
2. Reaction (a) would take place only if it is coupled to an exergonic reaction. Reaction (b) would proceed only if coupled to an exergonic reaction. Reaction (c) would proceed as written.
3. The information given here deals with the thermodynamics of the reaction, not the kinetics. It is not possible to predict the rate of the reaction.

15.2 A Modified Standard State for Biochemical Applications

4. The usual thermodynamic standard state refers to pH = 0. This is not very useful in biochemistry.
5. Statement (a) is true, but statement (b) is not. The standard state of solutes is normally defined as unit activity (1 *M* for all but the most careful work). In biological systems, the pH is frequently in the neutral range (i.e., H^+ is close to 10^{-7} *M*); the modification is a matter of convenience. Water is the solvent, not a solute, and its standard state is the pure liquid.
6. The designation $\Delta G^{\circ\prime}$ indicates a biological standard state. If the prime is omitted, then it is for chemical standard states.
7. No, there is no relationship between the thermodynamic quantity ΔG° and the speed. The ΔG° reflects the thermodynamic possibility under standard states. Speed is a kinetic quantity that is based on the ability of an enzyme to catalyze the reaction and the real substrate concentrations in the cell.
8. Assuming one significant figure, 20 kJ mol^{-1}, 0 kJ mol^{-1}, +30 kJ mol^{-1}.
9. $\Delta G^{\circ\prime} = \Delta H^{\prime} - T\Delta S^{\circ\prime}$ and $\Delta S^{\circ\prime}$ = 34.9 J mol^{-1} K^{-1} = 8.39 cal mol^{-1} K^{-1}. There are two particles on the reactant side of the equation and three on the product side, representing an increase in disorder.
10. Assuming 298 K and one significant figure:
    (a) –50 kJ
    (b) –20 kJ
    (c) –20 kJ
11. The levels of substrates and products can affect the true ΔG of a reaction, changing it from zero to a high number as in part (a). ΔG is negative when there is a larger amount of substrate than product.
12. The overall $\Delta G^{\circ\prime}$ = –260.4 kJ mol^{-1} or –62.3 kcal mol^{-1}. The reaction is exergonic, because it has a large, negative $\Delta G^{\circ\prime}$.
13. Greater than 3333 to 1.
14. Reaction (a) will not proceed as written; $\Delta G^{\circ\prime}$ = +12.6 kJ. Reaction (b) will proceed as written; $\Delta G^{\circ\prime}$ = –20.8 kJ. Reaction (c) will not proceed as written; $\Delta G^{\circ\prime}$ = +31.4 kJ. Reaction (d) will proceed as written; $\Delta G^{\circ\prime}$ = –18.0 kJ.
15. Yes, *if* you correct for the difference in temperature and concentrations from the standard values.
16. Two aspects are involved here. (a) Very rarely, if ever, are in vivo concentrations standard concentrations; actual ΔG (not ΔG°) values are very dependent on local concentrations, especially if the number of reactant molecules and product molecules is not the same. (b) Values of ΔG° rigorously apply only to *closed* systems that can reach equilibrium. Biochemical systems, however, are *open* systems and do not reach equilibrium. If you were at equilibrium, you would be dead. Metabolic pathways involve series of reactions, and the metabolic pathways themselves are interconnected, including processes that take in materials from the surroundings and release waste products to the surroundings.

15.3 The Nature of Metabolism

17. Group 1: catabolism, oxidative, energy-yielding. Group 2: anabolism, reductive, energy-requiring.
18. The local decrease in entropy associated with living organisms is balanced by the increase in the entropy of the surroundings caused by their presence. Coupling of reactions leads to overall dispersal of energy in the Universe.
19. The synthesis of sugars by plants in photosynthesis is endergonic and requires light energy from the Sun.
20. The biosynthesis of proteins is endergonic and is accompanied by a large decrease in entropy.
21. The ATP constantly generated by living organisms is used as a source of chemical energy for endergonic processes. There is a good deal of turnover of molecules, but no net change.

15.4 The Role of Oxidation and Reduction in Metabolism

22. (a) NADH is oxidized, $H^+ + NADH \rightarrow NAD^+ + 2e^- + 2H^+$. The aldehyde is reduced, $CH_3CH_2CHO + 2e^- + 2H^+ \rightarrow CH_3CH_2CH_2OH$.
    (b) Fe^{2+} is oxidized, $Fe^{2+} \rightarrow Fe^{3+} + e^-$. Cu^{2+} is reduced, $Cu^{2+} + e^- \rightarrow Cu^+$.
23. (a) The aldehyde is the oxidizing agent; NADH is the reducing agent.
    (b) Cu^{2+} is the oxidizing agent; Fe^{2+} is the reducing agent.

15.5 Coenzymes in Biologically Important Oxidation–Reduction Reactions

24. NAD^+, $NADP^+$, and FAD all contain an ADP moiety.
25. In NADPH, the 2′ hydroxyl of the ribose attached to the adenine has a phosphate attached.
26. There is little effect in the reactions. Both are coenzymes involved in oxidation–reduction reactions. The presence of the phosphate distinguishes two separate pools of coenzymes so that different ratios of NADPH/$NADP^+$ versus NADH/NAD^+ can be maintained.
27. None of these statements is true. Some coenzymes are involved in group-transfer reactions (recall this from Chapter 7). Many coenzymes contain phosphate groups, and CoA contains sulfur. ATP does not represent stored energy, but is generated on demand.
28. Redox reactions. NAD^+, or NADPH in an anabolic process, would likely be used. FAD probably would not be used because its free-energy change is too low.
29. The second half reaction (the one involving NADH) is that of oxidation; the first half reaction (the one involving O_2) is that of reduction. The overall reaction is $\frac{1}{2} O_2 + NADH + H^+ \rightarrow H_2O + NAD^+$. O_2 is the oxidizing agent and NADH is the reducing reagent.
30. See Figures 15.3 and 15.4.
31. Glucose-6-phosphate is oxidized, and $NADP^+$ is reduced. $NADP^+$ is the oxidizing agent, and glucose-6-phosphate is the reducing agent.
32. FAD is reduced, and succinate is oxidized. FAD is the oxidizing agent, and succinate is the reducing agent.
33. It is important to have two different pools of redox coenzymes. In the cytosol, the NAD^+/NADH ratio is high, but the NADPH/$NADP^+$ ratio is also high. This means that anabolic reactions can take place in the cytosol, while catabolic reactions, such as glycolysis, can also take place. If there were not two different pools of these coenzymes, no single cell location could have both catabolism and anabolism. Having two different, but structurally related, reducing agents helps keep anabolic and catabolic reactions distinct from each other.

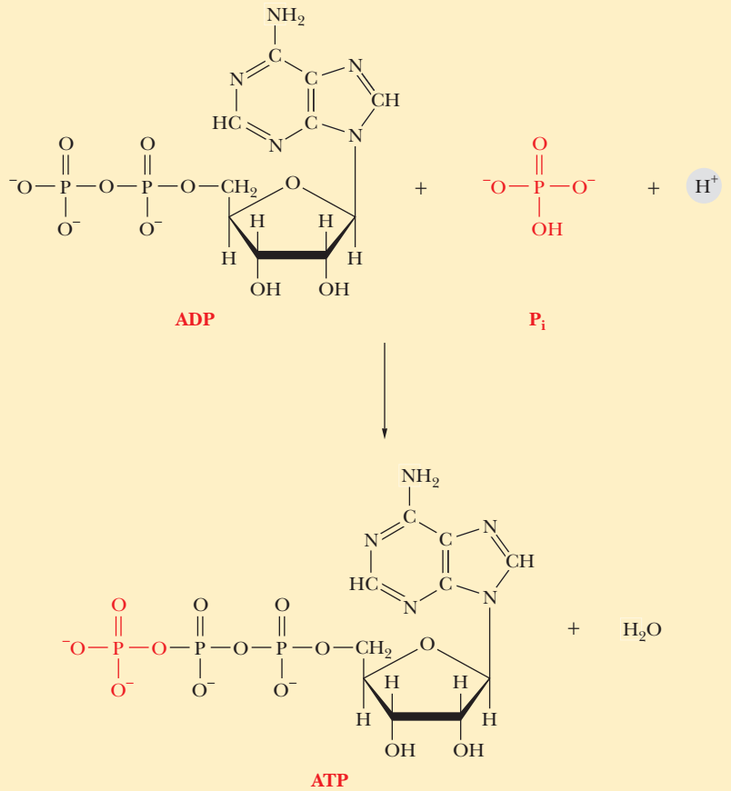
15.6 Coupling of Production and Use of Energy

34. The ratio of substrates to products would have to be 321,258 to 1.
35. Creatine phosphate + ADP → Creatine + ATP;
    $\Delta G^{\circ\prime} = -12.6$ kJ
    ATP + Glycerol → ADP + Glycerol-3-phosphate;
    $\Delta G^{\circ\prime} = -20.8$ kJ
    Creatine phosphate + Glycerol →
    Creatine + Glycerol-3-phosphate;
    $\Delta G^{\circ\prime}$ overall = −33.4 kJ
36. Glucose-l-phosphate → Glucose + P_i;
    $\Delta G^{\circ\prime} = -20.9$ kJ mol^{-1}
    Glucose + P_i → Glucose-6-phosphate;
    $\Delta G^{\circ\prime} = +12.5$ kJ mol^{-1}
    Glucose-1-phosphate → Glucose 6-phosphate;
    $\Delta G^{\circ\prime} = -8.4$ kJ mol^{-1}
37. In both pathways, the overall reaction is ATP + 2 H_2O →
    AMP + 2 P_i. Thermodynamic parameters, such as energy, are additive. The overall energy is the same because the overall pathway is the same.
38. Phosphoarginine + ADP → Arginine + ATP;
    $\Delta G^{\circ\prime} = -1.7$ kJ
    ATP + H_2O → ADP + P_i;
    $\Delta G^{\circ\prime} = -30.5$ kJ
    Phosphoarginine + H_2O → Arginine + P_i;
    $\Delta G^{\circ\prime} = -32.2$ kJ

39. ATP is less stable than ADP and P_i because of the charge distribution and loss of the resonance stabilization in the phosphate ion. There is stabilization (dispersal of energy) when ATP is hydrolyzed, leading to a negative free-energy change.

ADP + P_i + H^+ ⟶ ATP + H_2O $\quad \Delta G^{\circ\prime} = 30.5\ \text{kJ mol}^{-1} = 7.3\ \text{kcal mol}^{-1}$

or in structural form,



40. It is intermediate; thus, ATP is ideally positioned to serve as a phosphate donor or (as ADP) a phosphate acceptor, depending on local concentrations.
41. Creatine phosphate can phosphorylate ADP to ATP. There is a biochemical "germ of truth" here, but the effectiveness of such a supplement is another matter.
42. There is a large increase in entropy accompanying the hydrolysis of one molecule to five separate molecules.
43. PEP is a high-energy compound because energy is released upon its hydrolysis, owing to the resonance stabilization of the inorganic phosphate released and the possible keto–enol tautomerization of its product, pyruvate. See Figure 15.8.
44. The fact that a reaction is thermodynamically favorable does not mean that it will occur biologically. Even though there appears to be ample energy to catalyze the production of 2 ATPs from PEP, there is no enzyme that catalyzes this reaction.
45. Sprints and similar short periods of exercise rely on anaerobic metabolism as a source of energy, producing lactic acid. Longer periods of exercise also draw on aerobic metabolism.

15.7 Coenzyme A in Activation of Metabolic Pathways

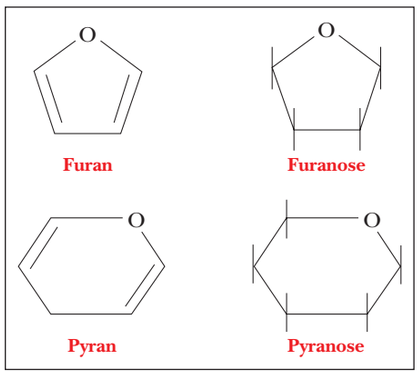
46. An activation step leads to an exergonic next step in a pathway. It is similar to the way in which organic chemists want to attach a good leaving group for the next step in a series of reactions.
47. Small energy changes generally involve mild conditions. Also, such reactions are more sensitive to relatively small changes in concentration and thus are easier to control.
48. Thioesters are high-energy compounds. The possible dissociation of the products after hydrolysis and resonance structures of the products facilitate reaction.
49. Coenzyme A serves several purposes. It is a high-energy compound, activating the initial steps of the metabolic pathway. It is used as a tag to "earmark" a molecule for a particular pathway. It is large and cannot cross membranes, so compartmentalization of pathways can be affected by binding metabolites to coenzyme A.
50. The size and complexity of the molecule make it more specific for particular enzyme-catalyzed reactions. In addition, it cannot cross membranes, so acyl-CoA molecules and other CoA derivatives can be segregated.

Chapter 16

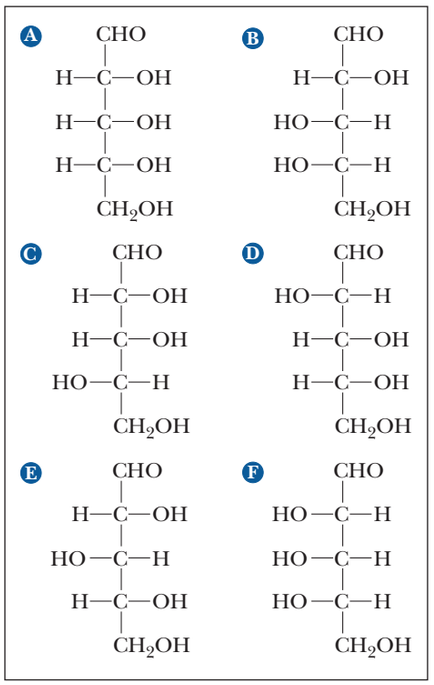
16.1 Sugars: Their Structures and Stereochemistry

1. A polysaccharide is a polymer of simple sugars, which are compounds that contain a single carbonyl group and several hydroxyl groups. A furanose is a cyclic sugar that contains a five-membered ring similar to that in furan. A pyranose is a cyclic sugar that contains a six-membered ring similar to that in pyran. An aldose is a sugar that contains an aldehyde group; a ketose is a sugar that contains a ketone group. A glycosidic bond is the acetal link-

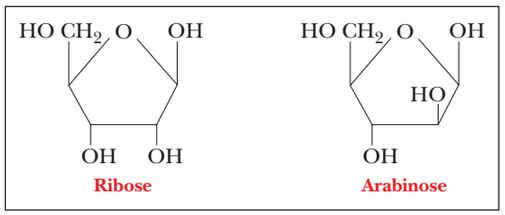
age that joins two sugars. An oligosaccharide is a compound formed by the linking of several simple sugars (monosaccharides) by glycosidic bonds. A glycoprotein is formed by the covalent bonding of sugars to a protein.



2. D-Mannose and D-galactose are both epimers of D-glucose, with inversion of configuration around carbon atoms 2 and 4, respectively; D-ribose has only five carbons, but the rest of the sugars named in this question have six.
3. All groups are aldose–ketose pairs.
4. Enantiomers are nonsuperimposable, mirror-image stereoisomers. Diastereomers are nonsuperimposable, nonmirror-image stereoisomers.
5. Four epimers of D-glucose exist, with inversion of configuration at a single carbon. The possible carbons at which this is possible are those numbered two through five.
6. Furanoses and pyranoses have five-membered and six-membered rings, respectively. It is well known from organic chemistry that rings of this size are the most stable and the most readily formed.
7. There are four chiral centers in the open-chain form of glucose (carbons two through five). Cyclization introduces another chiral center at the carbon involved in hemiacetal formation, giving a total of five chiral centers in the cyclic form.
8. Enantiomers: (a) and (f), (b) and (d). Epimers: (a) and (c), (a) and (d), (a) and (e), (b) and (f).



9. L-Sorbitol was named early in biochemical history as a derivative of L-sorbose. Reduction of D-glucose gives a hydroxy sugar that could easily be named D-glucitol, but it was originally named L-sorbitol and the name stuck.
10. Arabinose is an epimer of ribose. Nucleosides in which arabinose is substituted for ribose act as inhibitors in reactions of ribonucleosides.



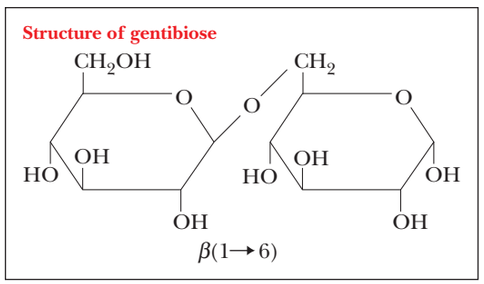
11. Converting a sugar to an epimer requires inversion of configuration at a chiral center. This can be done only by breaking and re-forming covalent bonds.
12. Two different orientations with respect to the sugar ring are possible for the hydroxyl group at the anomeric carbon. The two possibilities give rise to the new chiral center.

16.2 Reactions of Monosaccharides

13. This compound contains a lactic acid side chain.
14. In a sugar phosphate, an ester bond is formed between one of the sugar hydroxyls and phosphoric acid. A glycosidic bond is an acetal, which can be hydrolyzed to regenerate the two original sugar hydroxyls.
15. A reducing sugar is one that has a free aldehyde group. The aldehyde is easily oxidized, thus reducing the oxidizing agent.
16. Vitamin C is a lactone (a cyclic ester) with a double bond between two of the ring carbons. The presence of the double bond makes it susceptible to air oxidation.

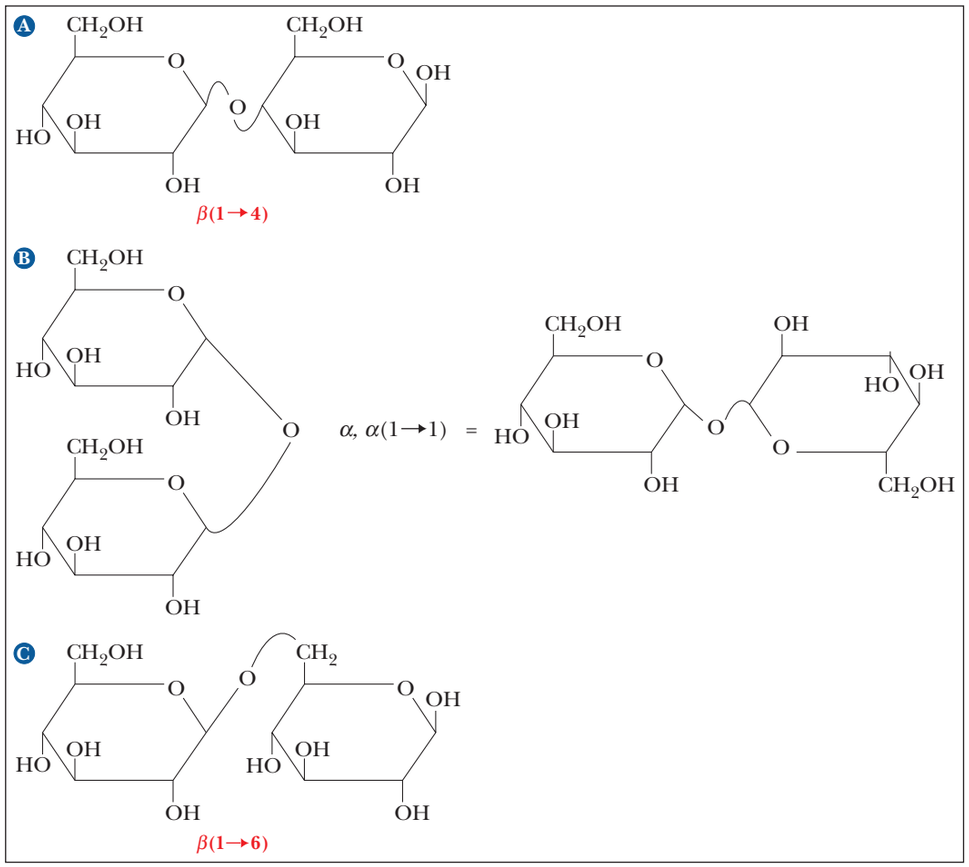
16.3 Some Important Oligosaccharides

17. Similarities: sucrose and lactose are both disaccharides, and both contain glucose. Differences: sucrose contains fructose, whereas lactose contains galactose. Sucrose has an $\alpha,\beta(1 \rightarrow 2)$ glycosidic linkage, whereas lactose has a $\beta(1 \rightarrow 4)$ glycosidic linkage.
18.



19. In some cases, the enzyme that degrades lactose (milk sugar) to its components—glucose and galactose—is missing. In other cases, the enzyme isomerizes galactose to glucose for further metabolic breakdown.

20.

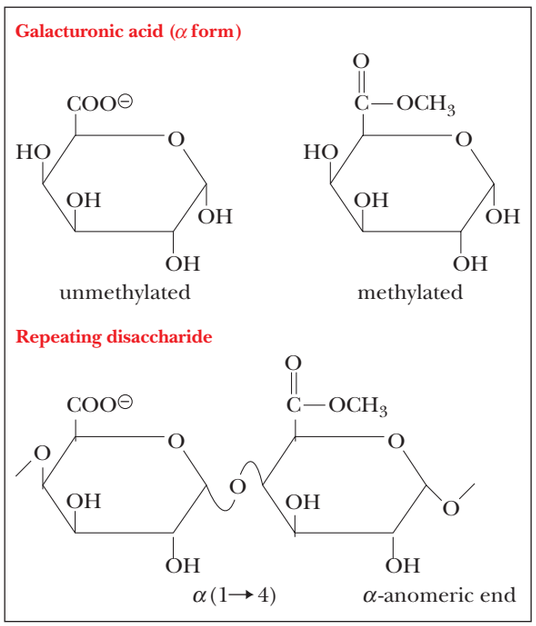


21. Milk contains lactose. Many people are sensitive to lactose and require an alternative beverage.

16.4 Structures and Functions of Polysaccharides

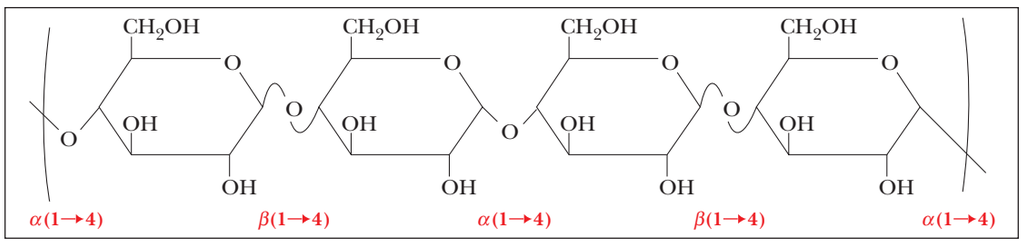
22. The cell walls of plants consist mainly of cellulose, whereas those of bacteria consist mainly of polysaccharides with peptide crosslinks.
23. Chitin is a polymer of *N*-acetyl-β-D-glucosamine, whereas cellulose is a polymer of D-glucose. Both polymers play a structural role, but chitin occurs in the exoskeletons of invertebrates and cellulose primarily in plants.
24. Glycogen and starch differ mainly in the degree of chain branching. Both polymers serve as vehicles for energy storage, glycogen in animals and starch in plants.
25. Both cellulose and starch are polymers of glucose. In cellulose, the monomers are joined by a β-glycosidic linkage, whereas in starch they are joined by an α-glycosidic linkage.
26. Glycogen exists as a highly branched polymer. Starch can have both a linear and a branched form, which is not as highly branched as that of glycogen.
27. Plant cell walls consist almost exclusively of carbohydrates, whereas bacterial cell walls contain peptides.

28. Repeating disaccharide of pectin:



29. Glucose and fructose.
30. Differences in structure: cellulose consists of linear fibers, but starch has a coil form. Differences in function: cellulose has a structural role, but starch is used for energy storage.
31. The concentration of reducing groups is too small to detect.
32. To 2500, one place (0.02%). To 1000, four places (0.08%). To 200, 24 places (0.48%).

33. This polymer would be expected to have a structural role. The presence of the β-glycosidic linkage makes it useful as food only to termites or to ruminants, such as cows and horses; these animals harbor bacteria capable of attacking the β-linkage in their digestive tracts.



34. Because of the branching, the glycogen molecule gives rise to a number of available glucose molecules at a time when it is being hydrolyzed to provide energy. A linear molecule could produce only one available glucose at a time.
35. The digestive tract of these animals contains bacteria that have the enzyme to hydrolyze cellulose.
36. Humans lack the enzyme to hydrolyze cellulose. In addition, the fibrous structure of cellulose makes it too insoluble to digest, even if humans had the necessary enzyme.
37. The enzyme β-amylase is an exoglycosidase, degrading polysaccharides from the ends. The enzyme α-amylase is an endoglycosidase, cleaving internal glycosidic bonds.
38. Fiber binds many toxic substances in the gut and decreases the transit time of ingested food in the digestive tract, so that harmful compounds such as carcinogens are removed from the body more quickly than would be the case with a low-fiber diet.
39. A cellulase (an enzyme that degrades cellulose) needs an active site that can recognize glucose residues joined in a β-glycosidic linkage and hydrolyze that linkage. An enzyme that degrades starch has the same requirements with regard to glucose residues joined in an α-glycosidic linkage.
40. Cross-linking can be expected to play a role in the structures of polysaccharides where mechanical strength is an issue. Examples include cellulose and chitin. These crosslinks can be readily formed by extensive hydrogen bonding. (See Figure 16.19.)
41. The sequence of monomers in a polysaccharide is not genetically coded, and, in this sense, it does not contain information.
42. It can be useful for polysaccharides to have a number of ends, characteristic of a branched polymer, rather than the two ends of a linear polymer. This would be the case when it is necessary to release residues from the ends as quickly as possible. Polysaccharides achieve this by having $1 \rightarrow 4$ and $1 \rightarrow 6$ glycosidic linkages to a residue at a branch point.
43. Chitin is a suitable material for the exoskeleton of invertebrates because of its mechanical strength. Individual polymer strands are cross-linked by hydrogen bonding, accounting for the strength. Cellulose is another polysaccharide cross-linked in the same way, and it can play a similar role.
44. Bacterial cell walls are not likely to consist largely of protein. Polysaccharides are easily formed and confer considerable mechanical strength. They are likely to play a large role.
45. Athletes try to increase their stores of glycogen before an event. The most direct way to increase the amount of this polymer of glucose is to eat carbohydrates.
46. Iodine is the reagent that will be added to the reaction mixture in the titration. When the end point is reached, the next drop of iodine will produce a characteristic blue color in the presence of the indicator.
47. Heparin is an anticoagulant. Its presence prevents blood clotting.
48. Glycosidic bonds can be formed between the side-chain hydroxyls of serine or threonine residues and the sugar hydroxyls. In addition, there is the possibility of ester bonds forming between the side-chain carboxyl groups of aspartate or glutamate and the sugar hydroxyls.

16.5 Glycoproteins

49. Glycoproteins are ones in which carbohydrates are covalently bonded to proteins. They play a role in eukaryotic cell membranes, frequently as recognition sites for external molecules. Antibodies (immunoglobulins) are glycoproteins.
50. The sugar portions of the blood-group glycoproteins are the source of the antigenic difference.

Chapter 17

17.1 The Overall Pathway in Glycolysis

1. Reactions that require ATP: phosphorylation of glucose to give glucose-6-phosphate and phosphorylation of fructose-6-phosphate to give fructose-1,6-*bis*phosphate. Reactions that produce ATP: transfer of phosphate group from 1,3-*bis*phosphoglycerate to ADP and transfer of phosphate group from phosphoenolpyruvate to ADP. Enzymes that catalyze reactions requiring ATP: hexokinase, glucokinase, and phosphofructokinase. Enzymes that catalyze reactions producing ATP: phosphoglycerate kinase and pyruvate kinase.
2. Reactions that require NADH: reduction of pyruvate to lactate and reduction of acetaldehyde to ethanol. Reactions that require NAD^+: oxidation of glyceraldehyde-3-phosphate to give 1,3-diphosphoglycerate. Enzymes that catalyze reactions requiring NADH: lactate dehydrogenase and alcohol dehydrogenase. Enzymes that catalyze reactions requiring NAD^+: glyceraldehyde-3-phosphate dehydrogenase.
3. Pyruvate can be converted to lactate, ethanol, or acetyl-CoA.

17.2 Conversion of Six-Carbon Glucose to Three-Carbon Glyceraldehyde-3-Phosphate

4. Aldolase catalyzes the reverse aldol condensation of fructose-1,6-*bis*phosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.
5. Isozymes are oligomeric enzymes that have slightly different amino acid compositions in different organs. Lactate dehydrogenase is an example, as is phosphofructokinase.
6. Isozymes allow for subtle control of the enzyme to respond to different cellular needs. For example, in the liver, lactate dehydrogenase is most often used to convert lactate to pyruvate, but the reaction is often reversed in the muscle. Having a different isozyme in the muscle and liver allows for those reactions to be optimized.
7. Fructose-1,6-*bis*phosphate can only undergo the reactions of glycolysis. The components of the pathway up to this point can have other metabolic fates.
8. Add the $\Delta G^{\circ\prime}$ mol^{-1} values for the reactions from glucose to glyceraldehyde-3-phosphate. The result is 2.5 kJ mol^{-1} = 0.6 kcal mol^{-1}.
9. The two enzymes can have different tissue locations and kinetic parameters. The glucokinase has a higher K_M for glucose than hexokinase. Thus, under conditions of low glucose, the liver does not convert glucose to glucose-6-phosphate, using the substrate that is needed elsewhere. When the glucose concentration is much higher, however, glucokinase helps phosphorylate glucose so that it can be stored as glycogen.
10. Individuals who lack the gene that directs the synthesis of the M form of the enzyme can carry on glycolysis in their livers but experience muscle weakness because they lack the enzyme in muscle.
11. The hexokinase molecule changes shape drastically on binding to substrate, consistent with the induced-fit theory of an enzyme adapting itself to its substrate.
12. ATP inhibits phosphofructokinase, consistent with the fact that ATP is produced by later reactions of glycolysis.

17.3 Glyceraldehyde-3-Phosphate Is Converted to Pyruvate

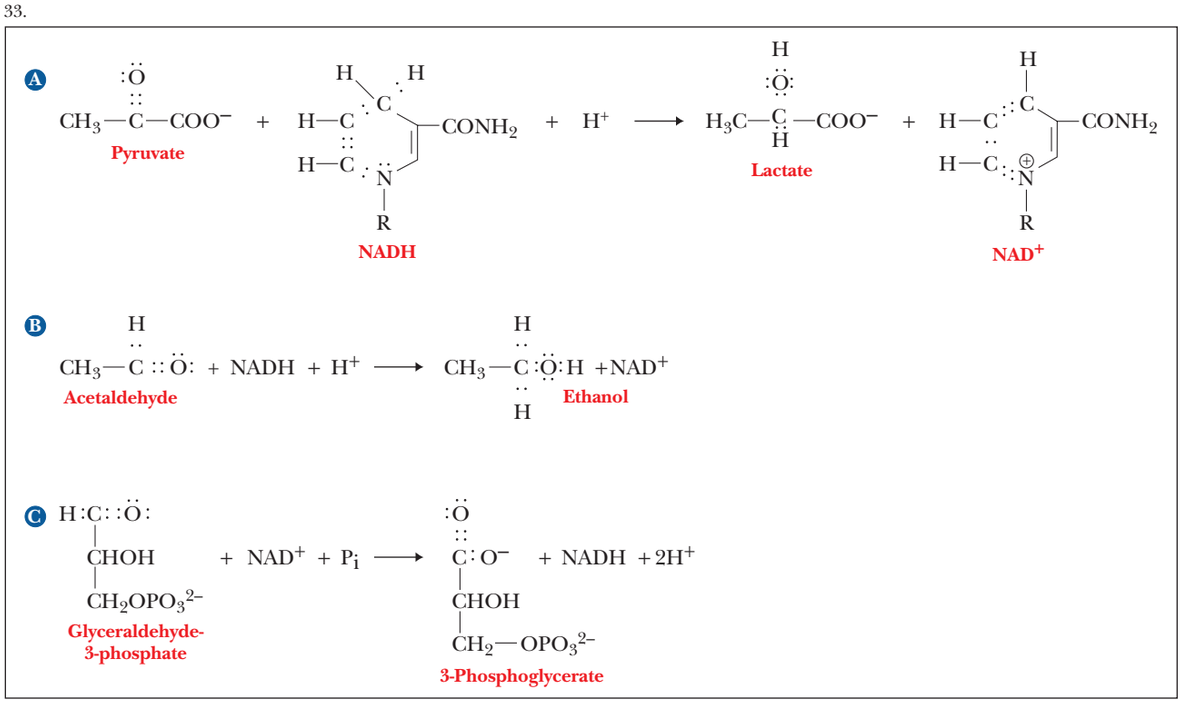
13. From the point at which aldolase splits fructose-1,6-*bis*phosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate; all reactions of the pathway are doubled (only the path from one glyceraldehyde-3-phosphate is usually shown).
14. NADH-linked dehydrogenases: Glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase, and alcohol dehydrogenase.

15. The free energy of hydrolysis of a substrate is the energetic driving force in substrate-level phosphorylation. An example is the conversion of glyceraldehyde-3-phosphate to 1,3-*bis*phosphoglycerate.
16. The control points in glycolysis are the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.
17. Hexokinase is inhibited by glucose-6-phosphate. Phosphofructokinase is inhibited by ATP and citrate. Pyruvate kinase is inhibited by ATP, acetyl-CoA, and alanine. Phosphofructokinase is stimulated by AMP and fructose-2,6-*bis*phosphate. Pyruvate kinase is stimulated by AMP and fructose-1,6-*bis*phosphate.
18. The part of the active site that binds to NADH would be the part that is most conserved, since many dehydrogenases use that coenzyme.
19. (a) Using a high-energy phosphate to phosphorylate a substrate.
    (b) Changing the form of a molecule without changing its empirical formula (i.e., replacing one isomer with another).
    (c) Performing an aldol cleavage of a sugar to yield two smaller sugars or sugar derivatives.
    (d) Changing the oxidation state of a substrate by removing hydrogens while simultaneously changing the oxidation state of a coenzyme (NADH, $FADH_2$, etc.).
20. An isomerase is a general term for an enzyme that changes the form of a substrate without changing its empirical formula. A mutase is an enzyme that moves a functional group, such as a phosphate, to a new location in a substrate molecule.
21. The reaction of 2-phosphoglycerate to phosphoenolpyruvate is a dehydration (loss of water) rather than a redox reaction.
22. Carbon-1 of glyceraldehyde is the aldehyde group. It changes oxidation state to a carboxylic acid, which is phosphorylated simultaneously.
23. ATP is an inhibitor of several steps of glycolysis as well as other catabolic pathways. The purpose of catabolic pathways is to produce energy, and high levels of ATP mean the cell already has sufficient energy. Glucose-6-phosphate inhibits hexokinase and is an example of product inhibition. If the glucose-6-phosphate level is high, it may indicate that sufficient glucose is available from glycogen breakdown or that the subsequent enzymatic steps of glycolysis are going slowly. Either way, there is no reason to produce more glucose-6-phosphate. Phosphofructokinase is inhibited by a special effector molecule, fructose-2,6-*bis*phosphate, whose levels are controlled by hormones. It is also inhibited by citrate, which indicates that there is sufficient energy from the citric acid cycle, probably from fat and amino acid degradation. Pyruvate kinase is also inhibited by acetyl-CoA, the presence of which indicates that fatty acids are being used to generate energy for the citric acid cycle. The main function of glycolysis is to feed carbon units to the citric acid cycle. When these carbon skeletons can come from other sources, glycolysis is inhibited to spare glucose for other purposes.
24. There would be 15 possible isozymes of LDH, combining three different subunits into combinations of four. Besides the five isozymes containing only M and H, there would also be C_4, CH_3, C_2H_2, C_3H, CH_2M, C_2HM, C_3M, CHM_2, C_2M_2, and CM_3.
25. Glutamic acid has an acidic side chain with a pK_a of 4.25. Therefore, it would be negatively charged at pH 8.6, and the H subunit would move more toward the anode (+) than the M subunit. Thus, LDH 1, which is H_4, would move the farthest. LDH 5, which is M_4, would move the least, with the other isozymes migrating between those two extremes proportional to their H content.
26. The formation of fructose-1,6-*bis*phosphate is the committed step in the glycolytic pathway. It is also one of the energy-requiring steps of the pathway.
27. Glucose-6-phosphate inhibits hexokinase, the enzyme responsible for its own formation. Because G-6-P is used up by additional reactions of glycolysis, the inhibition is relieved.
28. With few exceptions, a biochemical reaction typically results in only one chemical modification of the substrate. Accordingly, several to many steps are needed to reach the ultimate goal.
29. The enzyme contains a phosphate group on a suitable amino acid, such as serine, threonine, and histidine. The substrate donates its phosphate group from the C-3 position to another amino acid on the enzyme, subsequently receiving the one that started out on the enzyme. Thus, the ^{32}P that was on the substrate is transferred to the enzyme, while an unlabeled phosphorus is put on the C-2 position.

17.4 Anaerobic Metabolism of Pyruvate

30. The bubbles in beer are CO_2, produced by alcoholic fermentation. Tired and aching muscles are caused in part by a buildup of lactic acid, a product of anaerobic glycolysis.
31. The problem with lactic acid is that it is an acid. The H^+ produced from lactic acid formation causes the burning muscle sensation. Sodium lactate is the conjugate weak base of lactic acid. It is reconverted to glucose by gluconeogenesis in the liver. Giving sodium lactate intravenously is a good way to supply an indirect source of blood glucose.
32. The purpose of the step that produces lactic acid is to reduce pyruvate so that NADH can be oxidized to NAD^+, which is needed for the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

33.



34. Thiamine pyrophosphate is a coenzyme in the transfer of two-carbon units. It is required for catalysis by pyruvate decarboxylase in alcoholic fermentation.
35. The important part of TPP is the five-membered ring, in which a carbon is found between a nitrogen and a sulfur. This carbon forms a carbanion and is extremely reactive, making it able to perform a nucleophilic attack on carbonyl groups, leading to decarboxylation of several compounds in different pathways.
36. Thiamine pyrophosphate is a coenzyme required in the reaction catalyzed by pyruvate carboxylase. Because this reaction is a part of the metabolism of ethanol, less will be available to serve as a coenzyme in the reactions of other enzymes that require it.
37. Animals that have been run to death have accumulated large amounts of lactic acid in their muscle tissue, accounting for the sour taste of the meat.
38. Conversion of glucose to lactate rather than pyruvate recycles NADH.
39. This is possible, and it is done. These poisons also affect other tissues, including skin, hair, cells of the intestinal lining, and especially the immune system and red blood cells. People on chemotherapy are usually more susceptible to infectious diseases than healthy people and are often somewhat anemic.

17.5 Energy Production in Glycolysis

40. The energy released by all the reactions of glycolysis is 184.5 kJ mol glucose^{-1}. The energy released by glycolysis drives the phosphorylation of two ADP to ATP for each molecule of glucose, trapping 61.0 kJ mol glucose^{-1}. The estimate of 33% efficiency comes from the calculation $(61.0/184.5) \times 100 = 33\%$.
41. There is a net gain of two ATP molecules per glucose molecule consumed in glycolysis.
42. The gross yield is four ATP molecules per glucose molecule, but the reactions of glycolysis require two ATP per glucose.
43. The reactions catalyzed by hexokinase, phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerokinase, and pyruvate kinase.
44. The steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.
45. Phosphoenolpyruvate $\rightarrow$ pyruvate + P_i

    $\Delta G^{\circ\prime} = -61.9$ kJ mol^{-1} $= -14.8$ kcal mol^{-1}

    ADP + $P_i \rightarrow$ ATP

    $\Delta G^{\circ\prime} = 30.5$ kJ mol^{-1} $= 7.3$ kcal mol^{-1}

    Phosphoenolpyruvate + ADP $\rightarrow$ Pyruvate + ATP

    $\Delta G^{\circ\prime} = -31.4$ kJ mol^{-1} $= -7.5$ kcal mol^{-1}

46. The net yield of ATP from glycolysis is the same, two ATP, when either of the three substrates is used. The energetics of the conversion of hexoses to pyruvate are the same, regardless of hexose type.
47. Starting with glucose-1-phosphate, the net yield is three ATP, because one of the priming reactions is no longer used. Thus, glycogen is a more efficient fuel for glycolysis than free glucose.
48. Phosphoenolpyruvate + ADP $\rightarrow$ Pyruvate + ATP $\quad \Delta G^{\circ\prime} = -31.4$ kJ/mol

    ADP + $P_i \rightarrow$ ATP $\quad \Delta G^{\circ\prime} = 30.5$ kJ/mol

    Sum $\quad \Delta G^{\circ\prime} = -0.9$ kJ/mol

    Thus, the reaction is thermodynamically possible under standard conditions.
49. No, the reaction shown in Question 48 does not occur in nature. We can assume that no enzyme evolved that could catalyze it. Nature is not 100% efficient.
50. A positive $\Delta G^{\circ\prime}$ does not necessarily mean that the reaction has a positive ΔG. Substrate concentrations can make a negative ΔG out of a positive $\Delta G^{\circ\prime}$.
51. The entire pathway can be looked at as a large coupled reaction. Thus, if the overall pathway has a negative ΔG, an individual step may be able to have a positive ΔG, and the pathway can still continue.

Chapter 18

18.1 How Glycogen Is Produced and Degraded

1. These two pathways occur in the same cellular compartment, and, if both are on at the same time, a futile ATP hydrolysis cycle results. Using the same mechanism to turn them on/off or off/on is highly efficient.
2. In phosphorolysis, a bond is cleaved by adding the elements of phosphoric acid across that bond, whereas in hydrolysis, the cleavage takes place by adding the elements of water across the bond.
3. Glucose-6-phosphate is already phosphorylated. This saves one ATP equivalent in the early stages of glycolysis.
4. Each glucose residue is added to the growing glycogen molecule by transfer from UDPG.
5. Glycogen synthase is subject to covalent modification and to allosteric control. The enzyme is active in its phosphorylated form and inactive when dephosphorylated. AMP is an allosteric inhibitor of glycogen synthase, whereas ATP and glucose-6-phosphate are allosteric activators.
6. There is a net gain of three, rather than two, ATP when glycogen, not glucose, is the starting material of glycolysis.
7. It "costs" one ATP equivalent (UTP to UDP) to add a glucose residue to glycogen. In degradation, about 90% of the glucose residues do not require ATP to produce glucose-1-phosphate. The other 10% require ATP to phosphorylate glucose. On average, this is another 0.1 ATP. Thus, the overall "cost" is 1.1 ATP, compared with the three ATP that can be derived from glucose-6-phosphate by glycolysis.
8. The ATP cost is the same, but more than 30 ATP can be derived from aerobic metabolism.
9. Eating high-carbohydrate foods for several days before strenuous activity is intended to build up glycogen stores in the body. Glycogen will be available to supply required energy.
10. The disaccharide sucrose can be hydrolyzed to glucose and fructose, which can both be readily converted to glucose-1-phosphate, the immediate precursor of glycogen. This is not the usual form of "glycogen loading."
11. Probably not, because the sugar spike initially results in a rapid increase in insulin levels, which results in lowering blood glucose levels and increased glycogen storage in the liver.
12. The sprint is essentially anaerobic and produces lactate from glucose by glycolysis. Lactate is then recycled to glucose by gluconeogenesis.
13. It is unlikely that this finding will be confirmed by other researchers. The highly branched structure of glycogen is optimized for release of glucose on demand.
14. Each glucose residue added to a growing phosphate chain comes from uridine diphosphate glucose. The cleavage of the phosphate ester bond to the nucleoside diphosphate moiety supplies the needed energy.
15. The enzyme that catalyzes addition of glucose residues to a growing glycogen chain cannot form a bond between isolated glucose residues; thus we have the need for a primer.
16. The glycogen synthase reaction is exergonic overall because it is coupled to phosphate ester hydrolysis.
17. (a) Increasing the level of ATP favors both gluconeogenesis and glycogen synthesis.
    (b) Decreasing the level of fructose-1,6-*bis*phosphate would tend to stimulate glycolysis, rather than gluconeogenesis or glycogen synthesis.
    (c) Levels of fructose-6- phosphate do not have a marked regulatory effect on these pathways of carbohydrate metabolism.
18. "Going for the burn" in a workout refers to the sensation that accompanies lactic acid buildup. This in turn arises from anaerobic metabolism of glucose in muscle.
19. Sugar nucleotides are diphosphates. The net result is hydrolysis to two phosphate ions, releasing more energy and driving the addition of glucose residues to glycogen in the direction of polymerization.

18.2 Gluconeogenesis Produces Glucose from Pyruvate

20. Reactions that require acetyl-CoA: none. Reactions that require biotin: carboxylation of pyruvate to oxaloacetate.
21. Three reactions of glycolysis are irreversible under physiological conditions. They are the production of pyruvate and ATP from phosphoenolpyruvate, the production of fructose-1,6-*bis*phosphate from fructose-6-phosphate, and the production of glucose-6-phosphate from glucose. These reactions are bypassed in gluconeogenesis; the reactions of gluconeogenesis differ from those of glycolysis at these points and are catalyzed by different enzymes.
22. Biotin is the molecule to which carbon dioxide is attached to the process of being transferred to pyruvate. The reaction produces oxaloacetate, which then undergoes further reactions of gluconeogenesis.

23. In gluconeogenesis, glucose-6-phosphate is dephosphorylated to glucose (the last step of the pathway); in glycolysis, it isomerizes to fructose-6-phosphate (an early step in the pathway).
24. Of the three processes—glycogen formation, gluconeogenesis, and the pentose phosphate pathway—only one, gluconeogenesis, involves an enzyme that requires biotin. The enzyme in question is pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, an early step in gluconeogenesis.
25. The hydrolysis of fructose-1,6-*bis*phosphate is a strongly exergonic reaction. The reverse reaction in glycolysis, phosphorylation of fructose-6-phosphate, is irreversible because of the energy supplied by ATP hydrolysis.

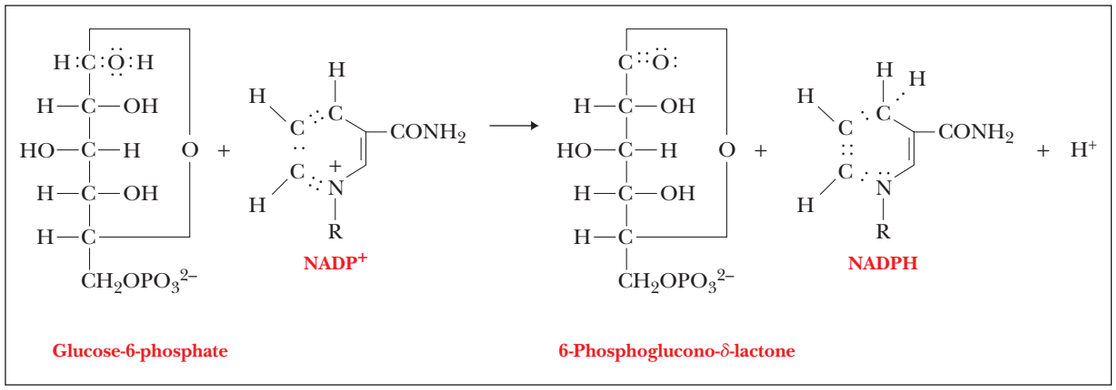
18.3 Control of Carbohydrate Metabolism

26. Reactions that require ATP: formation of UDP-glucose from glucose-1-phosphate and UTP (indirect requirement, because ATP is needed to regenerate UTP), regeneration of UTP, and carboxylation of pyruvate to oxaloacetate. Reactions that produce ATP: none. Enzymes that catalyze ATP-requiring reactions: UDP-glucose phosphorylase (indirect requirement), nucleoside phosphate kinase, and pyruvate carboxylase. Enzymes that catalyze ATP-producing reactions: none.
27. Fructose-2,6-*bis*phosphate is an allosteric activator of phosphofructokinase (a glycolytic enzyme) and an allosteric inhibitor of fructose *bis*phosphate phosphatase (an enzyme in the pathway of gluconeogenesis).
28. Hexokinase can add a phosphate group to any of several six-carbon sugars, whereas glucokinase is specific for glucose. Glucokinase has a lower affinity for glucose than does hexokinase. Consequently, glucokinase tends to deal with an excess of glucose, particularly in the liver. Hexokinase is the usual enzyme for phosphorylating six-carbon sugars.
29. The Cori cycle is a pathway in which there is cycling of glucose due to glycolysis in muscle and gluconeogenesis in liver. The blood transports lactate from muscle to liver and glucose from liver to muscle.
30. Substrate cycles are futile in the sense that there is no net change except for the hydrolysis of ATP. However, substrate cycles allow for increased control over opposing reactions when they are catalyzed by different enzymes.
31. Having two control mechanisms allows for fine-tuning of control and for the possibility of amplification. Both mechanisms are capable of rapid response to conditions, milliseconds in the case of allosteric control and seconds to minutes in the case of covalent modification.
32. Different control mechanisms have inherently different time scales. Allosteric control can take place in milliseconds, whereas covalent control takes seconds to minutes. Genetic control has a longer time scale than either.
33. The most important aspect of the amplification scheme is that the control mechanisms affect agents that are catalysts themselves. An enhancement by several powers of ten is itself increased by several powers of ten.
34. Enzymes, like all catalysts, speed up the forward and reverse reaction to the same extent. Having different catalysts is the only way to ensure independent control over the rates of the forward and reverse process.
35. Muscle tissue uses large quantities of glucose, producing lactate in the process. The liver is an important site of gluconeogenesis to recycle the lactate to glucose.
36. Fructose-2,6-*bis*phosphate is an allosteric activator of phosphofructokinase (a glycolytic enzyme) and an allosteric inhibitor of fructose *bis*phosphate phosphatase (an enzyme in the pathway of gluconeogenesis). It thus plays a role in two pathways that are not exactly the reverse of each other.
37. The concentration of fructose-2,6-*bis*phosphate in a cell depends on the balance between its synthesis (catalyzed by phosphofructokinase-2) and its breakdown (catalyzed by fructose *bis*phosphatase-2). The separate enzymes that control the formation and breakdown of fructose-2,6-*bis*phosphate are themselves controlled by a phosphorylation/dephosphorylation mechanism.
38. Glycogen is more extensively branched than starch. It is a more useful storage form of glucose for animals because the glucose can be mobilized more easily when there is a need for energy.

18.4 Glucose Is Sometimes Diverted through the Pentose Phosphate Pathway

39. NADPH has one more phosphate group than NADH (at the 2′ position of the ribose ring of the adenine nucleotide portion of the molecule). NADH is produced in oxidative reactions that give rise to ATP. NADPH is a reducing agent in biosynthesis. The enzymes that use NADH as a coenzyme are different from those that require NADPH.
40. Glucose-6-phosphate can be converted to glucose (gluconeogenesis), glycogen, pentose phosphates (pentose phosphate pathway), or pyruvate (glycolysis).
41. Hemolytic anemia is caused by defective working of the pentose phosphate pathway. There is a deficiency of NADPH, which indirectly contributes to the integrity of the red blood cells. The pentose phosphate pathway is the only source of NADPH in red blood cells.
42. (a) By using only the oxidative reactions.
    (b) By using the oxidative reactions, the transaldolase and transketolase reactions, and gluconeogenesis.
    (c) By using glycolytic reactions and the transaldolase and transketolase reactions in reverse.
43. Transketolase catalyzes the transfer of a two-carbon unit, whereas transaldolase catalyzes the transfer of a three-carbon unit.
44. In red blood cells, the presence of the reduced form of glutathione is necessary for the maintenance of the sulfhydryl groups of hemoglobin and other proteins in their reduced forms, as well as for keeping the Fe(II) of hemoglobin in its reduced form. Glutathione also maintains the integrity of red cells by reacting with peroxides that would otherwise degrade fatty-acid side chains in the cell membrane.
45. Thiamine pyrophosphate is a cofactor necessary for the function of transketolase, an enzyme that catalyzes one of the reactions in the nonoxidative part of the pentose phosphate pathway.

46.



47. Having different reducing agents for anabolic and catabolic pathways keeps the pathways separate metabolically. Thus, they are subject to independent control and do not waste energy.
48. If a cell needs NADPH, all the reactions of the pentose phosphate pathway take place. If a cell needs ribose-5-phosphate, the oxidative portion of the pathway can be bypassed; only the nonoxidative reshuffling reactions take place. The pentose phosphate pathway does not have a significant effect on the cell's supply of ATP.
49. The ester bond is more easily broken than any of the other bonds that form the sugar ring. Hydrolysis of that bond is the next step in the pathway.
50. The reshuffling reactions of the pentose phosphate pathway have both an epimerase and an isomerase. Without an isomerase, all the sugars involved are keto sugars, which are not substrates for transaldolase, one of the key enzymes in the reshuffling process.

Chapter 19

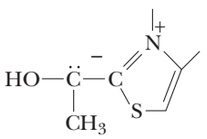
19.1 The Central Role of the Citric Acid Cycle in Metabolism

1. Anaerobic glycolysis is the principal pathway for the anaerobic metabolism of glucose. The pentose phosphate pathway can also be considered. Aerobic glycolysis and the citric acid cycle are responsible for the aerobic metabolism of glucose.
2. Anaerobically, two ATPs can be produced from one glucose molecule. Aerobically, this figure is 30 to 32, depending on in which tissue it is occurring.
3. The citric acid cycle is also called the Krebs cycle, the tricarboxylic acid cycle, and the TCA cycle.
4. Amphibolic means that the pathway is involved in both catabolism and anabolism.

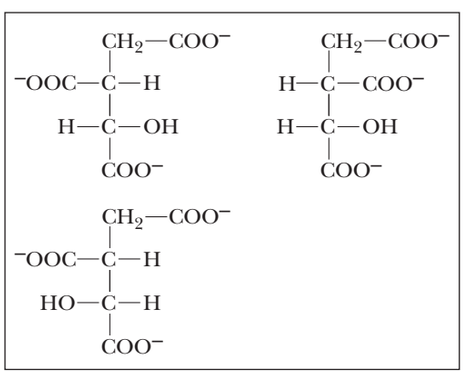
19.2 The Overall Pathway of the Citric Acid Cycle

5. The citric acid cycle takes place in the mitochondrial matrix. Glycolysis takes place in the cytosol.
6. There is a transporter on the inner mitochondrial matrix that allows pyruvate from the cytosol to pass into the mitochondria.
7. NAD^+ and FAD are the primary electron acceptors of the citric acid cycle.
8. NADH and $FADH_2$ are indirect sources of energy produced in the TCA cycle. GTP is a direct source of energy.

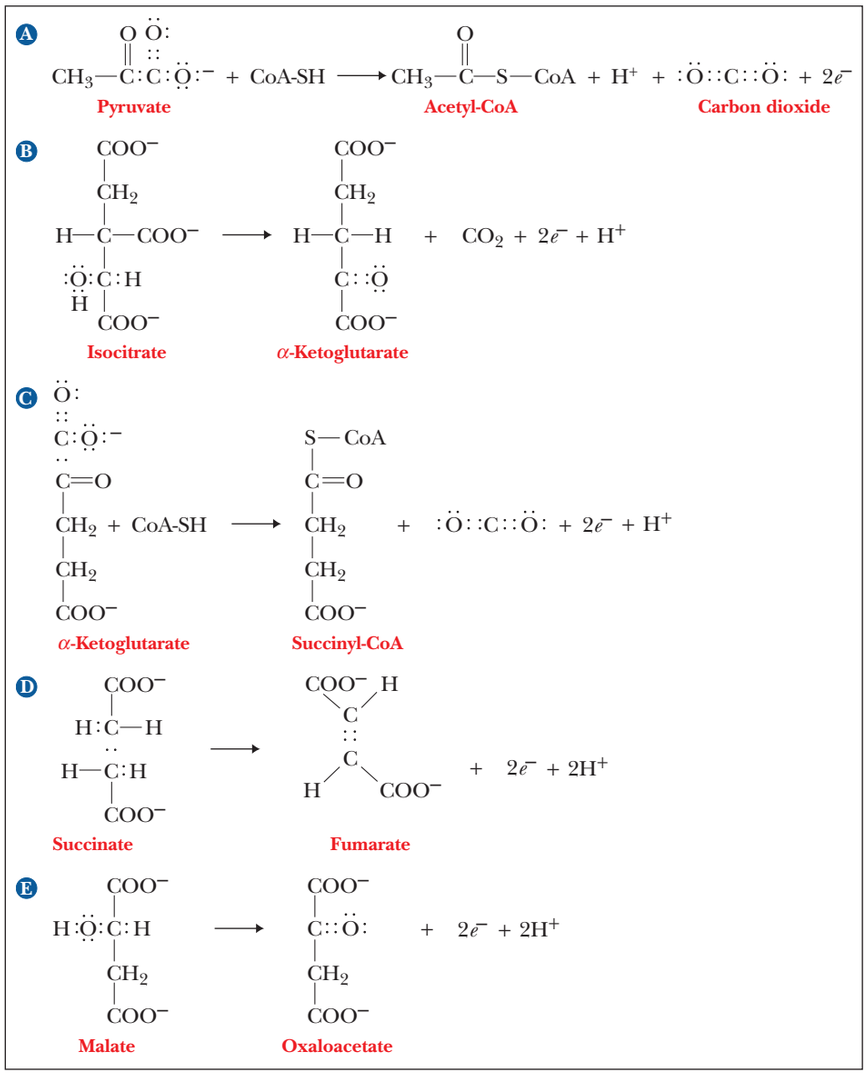
19.3 How Pyruvate Is Converted to Acetyl-CoA

9. Five enzymes are involved in the pyruvate dehydrogenase complex of mammals. Pyruvate dehydrogenase transfers a two-carbon unit to TPP and releases CO_2. Dihydrolipoyl transacetylase transfers the two-carbon acetyl unit to lipoic acid and then to coenzyme A. Dihydrolipoyl dehydrogenase reoxidizes lipoic acid and reduces NAD^+ to NADH. Pyruvate dehydrogenase kinase phosphorylates PDH. PDH phosphatase removes the phosphate.
10. Lipoic acid plays a role both in redox and in acetyl-transfer reactions.
11. Five enzymes are all in close proximity for efficient shuttling of the acetyl unit between molecules and efficient control of the complex by phosphorylation.
12. Thiamine pyrophosphate comes from the B vitamin thiamine. Lipoic acid is a vitamin. NAD^+ comes from the B vitamin niacin. FAD comes from the B vitamin riboflavin.
13. 
14. See Figure 19.4.

19.4 The Individual Reactions of the Citric Acid Cycle

15. A condensation reaction is one in which a new carbon–carbon bond is formed. The reaction of acetyl-CoA and oxaloacetate to produce citrate involves formation of such a carbon–carbon bond.
16. It means that the reaction catalyzed by the enzyme produces the product that is part of the name and does not require a direct input of energy from a high-energy phosphate. Thus, citrate synthase catalyzes the synthesis of citrate without using ATP to do it.
17. Fluoroacetate is a poison that is produced naturally in some plants and is also used as a poison against undesirable pests. It is poisonous because it is used by citrate synthase to make fluorocitrate, which is an inhibitor of the citric acid cycle.
18. The reaction involves an achiral molecule (citrate) being converted to a chiral one (isocitrate).
19. Conversion of pyruvate to acetyl-CoA, conversion of isocitrate to α-ketoglutarate, and conversion of α-ketoglutarate to succinyl-CoA.
20. Conversion of pyruvate to acetyl-CoA, conversion of isocitrate to α-ketoglutarate, conversion of α-ketoglutarate to succinyl-CoA, conversion of succinate to fumarate, and conversion of malate to oxaloacetate.
21. These enzymes catalyze oxidative decarboxylations.
22. The reactions proceed by the same mechanism and use the same cofactors. The difference is the initial substrate, which is pyruvate or α-ketoglutarate. During the course of the reaction, pyruvate dehydrogenase shuttles an acetyl unit through the reaction while α-ketoglutarate dehydrogenase shuttles a succinyl unit.
23. A synthetase is an enzyme that synthesizes a molecule and uses a high-energy phosphate in the process.
24. GTP is equivalent to ATP because an enzyme, nucleoside diphosphate kinase, is able to interconvert GTP and ATP.
25. The enzymes that reduce NAD^+ are all soluble, matrix enzymes, while succinate dehydrogenase is membrane-bound. The NAD^+-linked dehydrogenases all catalyze oxidations that involve carbons and oxygens, such as an alcohol group being oxidized to an aldehyde or aldehyde to carboxylic acid. The FAD-linked dehydrogenase oxidizes a carbon–carbon single bond to a double bond.
26. There is an adenine nucleotide portion in the structure of NADH, with a specific binding site on NADH-linked dehydrogenases for this portion of NADH.
27. The conversion of fumarate to malate is a hydration reaction, not a redox reaction.
28. 

29.



19.5 Energetics and Control of the Citric Acid Cycle

30. The reactions are catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.

31. PDH is controlled allosterically. It is inhibited by ATP, acetyl-CoA, and NADH. In addition, it is subject to control by phosphorylation. When PDH kinase phosphorylates PDH, it becomes inactive. Removing the phosphate with the PDH phosphatase reactivates it.

32. ATP and NADH are the two most common inhibitors.

33. If the amount of ADP in a cell increases relative to the amount of ATP, the cell needs energy (ATP). This situation not only favors the reactions of the citric acid cycle, which release energy, activating isocitrate dehydrogenase, but also stimulates the formation of NADH and $FADH_2$ for ATP production by electron transport and oxidative phosphorylation.

34. If the amount of NADH in a cell increases relative to the amount of NAD^+, the cell has completed a number of energy-releasing reactions. There is less need for the citric acid cycle to be active; as a result, the activity of pyruvate dehydrogenase is decreased.

35. The citric acid cycle is less active when a cell has a high ATP/ADP ratio and a high NADH/NAD^+ ratio. Both ratios indicate a high "energy charge" in the cell, indicating less of a need for the energy-releasing reactions of the citric acid cycle.

36. Thioesters are "high-energy" compounds that play a role in group-transfer reactions; consequently, their $\Delta G^{\circ\prime}$ of hydrolysis is large and negative to provide energy for the reaction.

37. The energy released by hydrolysis of acetyl-CoA is needed for the condensation reaction that links the acetyl moiety to oxaloacetate, yielding citrate. The energy released by hydrolysis of succinyl-CoA drives the phosphorylation of GDP, yielding GTP.

38. Table 19.2 shows that the sum of the energies of the individual reactions is –44.3 kJ (–10.6 kcal) for each mole of acetyl-CoA that enters the cycle.

39. The expression would relate to the intensive extraction of energy from intermediate compounds by redox reactions. Including the pyruvate dehydrogenase reaction, 5 of 9 reactions are redox reactions (in contrast with only 1 of 10 in glycolysis). Accordingly, energy is rapidly extracted from carbon compounds (yielding the energyless CO_2) and is transferred to NAD^+ and FAD for subsequent utilization.

40. Lactose is a disaccharide of glucose and galactose. There is no energy cost in the hydrolysis of the bond between the two monosaccharides, so essentially there are two hexoses to consider. Because the processing of any of the hexoses yields the same amount of energy, the aerobic processing of lactose would lead to 60 to 64 ATPs, depending on the tissue and on the shuttle system used.

19.6 The Glyoxylate Cycle: A Related Pathway

41. Isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and succinyl-CoA synthetase.

42. The conversion of isocitrate to succinate and glyoxylate catalyzed by isocitrate lyase and the conversion of glyoxylate and acetyl-CoA to malate catalyzed by malate synthase.

43. Bacteria that have a glyoxylate cycle can convert the acetic acid to amino acids, carbohydrates, and lipids, but humans can use the acetic acid only as an energy source or to make lipids.

19.7 The Citric Acid Cycle in Catabolism

44. The citric acid cycle is the central metabolic pathway and indirect producer of energy. It receives fuels from the other pathways at many points and generates reduced electron carriers that go into the electron transport chain. It is also involved in anabolism, as many of its intermediates can be drawn off to synthesize other compounds.
45. The citric acid cycle occurs in the mitochondrial matrix, which is more selective in its permeability than the plasma membrane.
46. In oxidative decarboxylation, the molecule that is oxidized loses a carboxyl group as carbon dioxide. Examples of oxidative decarboxylation include the conversion of pyruvate to acetyl-CoA, isocitrate to α-ketoglutarate, and α-ketoglutarate to succinyl-CoA.
47. Yes, not only is citric acid completely degraded to carbon dioxide and water, but it is also readily absorbed into the mitochondrion.

19.8 The Citric Acid Cycle in Anabolism

48. The following series of reactions exchanges NADH for NADPH.

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{Malate} + \text{NAD}^+$$

$$\text{Malate} + \text{NADP}^+ \rightarrow \text{Pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+$$

49. A variety of reactions in which amino acids are converted to citric acid cycle intermediates are considered anaplerotic. In addition, pyruvate + CO_2 can form oxaloacetate via pyruvate carboxylase.
50. Many compounds can form acetyl-CoA, such as fats, carbohydrates, and many amino acids. Acetyl-CoA can also form fats and ketone bodies, as well as feed directly into the citric acid cycle.

19.9 The Link to Oxygen

51. The NADH and $FADH_2$ produced by the citric acid cycle are the electron donors in the electron transport chain linked to oxygen. Because of this connection, the citric acid cycle is considered part of aerobic metabolism.

Chapter 20

20.1 The Role of Electron Transport in Metabolism

1. Electrons are passed from NADH to a flavin-containing protein to coenzyme Q. From coenzyme Q, the electrons pass to cytochrome *b*, then to cytochrome *c*, via the Q cycle, followed by cytochromes *a* and a_3. From the cytochrome aa_3 complex, the electrons are finally passed to oxygen.
2. Electron transport and oxidative phosphorylation are different processes. Electron transport requires the respiratory complexes of the inner mitochondrial membrane, whereas oxidative phosphorylation requires ATP synthase, also located on the inner mitochondrial membrane. Electron transport can take place in the absence of oxidative phosphorylation.
3. In all reactions, electrons are passed from the reduced form of one reactant to the oxidized form of the next reactant in the chain. The notation [Fe—S] refers to any one of a number of iron–sulfur proteins.

Reactions of Complex I

$$\textbf{NADH} + \textbf{E-FMN} \rightarrow \textbf{NAD}^+ + \textbf{E-FMNH}_2$$

$$\textbf{E-FMNH}_2 + \textbf{2[Fe—S]}_{ox} \rightarrow \textbf{E-FMN} + \textbf{2[Fe—S]}_{red}$$

Liberation of enough energy to produce ATP

Transfer to Coenzyme Q

$$\textbf{2[Fe—S]}_{red} + \textbf{CoQ} \rightarrow \textbf{2[Fe—S]}_{ox} + \textbf{CoQH}_2$$

Reactions of Complex III

Q cycle reactions

$$\textbf{[Fe—S]}_{red} + \textbf{cyt } c_{1ox} \rightarrow \textbf{[Fe—S]}_{ox} + \textbf{cyt } c_{2red}$$

Liberation of enough energy to produce ATP

Transfer to cytochrome *c*

$$\textbf{cyt } c_{2red} + \textbf{cyt } c_{ox} \rightarrow \textbf{cyt } c_{1ox} + \textbf{cyt } c_{red}$$

Reactions of Complex IV

$$\textbf{cyt } c_{red} + \textbf{cyt a-a}_{3ox} \rightarrow \textbf{cyt } c_{ox} + \textbf{cyt a-a}_{3red}$$

$$\textbf{cyt a-a}_{1red} + \tfrac{1}{2}\text{O}_2 \rightarrow \textbf{cyt a-a}_{10x} + \textbf{H}_2\textbf{O}$$

Liberation of enough energy to produce ATP

4. When $FADH_2$ is the starting point for electron transport, electrons are passed from $FADH_2$ to coenzyme Q in a reaction carried out by Complex II that bypasses Complex I.

$$\text{FADH}_2 + 2[\text{Fe—S}]_{ox} \rightarrow \text{FAD} + 2[\text{Fe—S}]_{red}$$

$$2[\text{Fe—S}]_{red} + \text{CoQ} \rightarrow 2[\text{Fe—S}]_{ox} + \text{CoQH}_2$$

5. Mitochondrial structure confines the reduced electron carriers produced by the citric acid cycle to the matrix. There they are close to the respiratory complexes of the electron transport chain that will pass the electrons from the carriers produced by the citric acid cycle to oxygen, the ultimate recipient of electrons and hydrogens.

20.2 Reduction Potentials in the Electron Transport Chain

6. The electron transport chain translocates charged particles by chemical means. Interconversion of chemical and electrical energy is exactly what a battery does.
7. The reactions are all written in the same direction for purposes of comparison. By convention, they are written as reduction, rather than oxidation, reactions.
8. $\Delta G^{\circ\prime} = -60$ kJ/mol
9. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$\text{NAD}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{NADH} + \text{H}^+$	−0.320

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ + 2\text{H}^+ + 2e^-$	0.820
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}$	0.816
$\text{NADH} + \text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$	1.136

10. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$\text{NAD}^+ + 2\text{H}^+ + 2e^- \rightarrow \text{NADH} + \text{H}^+$	−0.320

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ + 2\text{H}^+ + 2e^-$	0.820
$\text{Pyruvate} + 2\text{H}^+ + 2e^- \rightarrow \text{Lactate}$	−0.185
$\text{NADH} + \text{H}^+ + \text{Pyruvate} \rightarrow \text{NAD}^+ + \text{Lactate}$	0.135

11. We fundamentally add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
$\text{Fumarate} + 2\text{H}^+ + 2e^- \rightarrow \text{Succinate}$	0.031

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

$\text{Succinate} \rightarrow \text{Fumarate} + 2\text{H}^+ + 2e^-$	−0.031
$\frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}$	0.816
$\text{Succunate} + \frac{1}{2}\text{O}_2 \rightarrow \text{Fumarate} + \text{H}_2\text{O}$	0.785

12. The cytochrome is the electron donor, and the flavin moiety is the electron acceptor. Once again, we add the half reactions in Table 20.1.

	$E^{\circ\prime}$ (V)
Cytochrome $c(\text{Fe}^{3+}) + e^- \rightarrow$ Cytochrome $c(\text{Fe}^{2+})$	0.254

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

2 Cytochrome $c(\text{Fe}^{2+}) + 2e^- \rightarrow$ 2 Cytochrome $c(\text{Fe}^{3+})$	−0.254
$[\text{FAD}] + 2\text{ H}^+ + 2\ e^- \rightarrow [\text{FADH}_2]$	0.091
$[\text{FAD}] + 2\text{ Cyt } c(\text{Fe}^{2+}) + 2\text{H}^+ \rightarrow [\text{FADH}_2] + 2\text{ Cyt } c(\text{Fe}^{3+})$	−0.068

This was the maximum value for a bound flavin. The negative sign indicates that this reaction will not take place as written because it is not energetically favorable.

13. Here is an illustration based on standard reduction potentials.

	$E^{\circ\prime}$ (V)
$\text{Fumarate} + 2\text{H}^+ + 2e^- \rightarrow \text{Succinate}$	0.081

This is the wrong direction, so we reverse the equation and the sign of the potential difference.

Succinate → Fumarate + $2H^+ + 2e^-$	−0.081
FAD + $2H^+ + 2e^-$ → $FADH_2$	−0.219
Succinate + FAD → Fumarate + $FADH_2$	−0.250

The other possibility can be calculated in the same way.

Succinate → Fumarate + $2H^+ + 2e^-$	−0.081
$NAD^+ + 2H^+ + 2e^-$ + NADH + H^+	0.320
Succinate + NAD^+ → Fumarate + NADH	−0.851

Both reduction potentials indicate a reaction that is not energetically favorable, but less so with FAD than with NAD^+. Other factors enter into consideration, however, in a living cell. The first is that the reactions do not take place under standard conditions, altering the values of reduction potentials. The second is that the reduced electron carriers (NADH and $FADH_2$) are reoxidized. Coupling the reactions we have looked at here to others also makes them less unfavorable.

14. The half reaction of oxidation NADH + H^+ → NAD^+ + $2H^+$ + $2e^-$ is strongly exergonic ($\Delta G^{\circ\prime}$ = –61.3 kJ mol^{-1} = –14.8 kcal mol^{-1}), as is the overall reaction Pyruvate + NADH + H^+ → Lactate + NAD^+ ($\Delta G^{\circ\prime}$ = –25.1 kJ mol^{-1} = –6.0 kcal mol^{-1}).

20.3 Organization of Electron Transport Complexes

15. They all contain the heme group, with minor differences in the heme side chains in most cytochromes.
16. Cytochromes are proteins of electron transport; the heme ion alternates between the Fe(II) and Fe(III) states. The function of hemoglobin and myoglobin is oxygen transport and storage, respectively. The iron remains in the Fe(II) state.
17. Coenzyme Q is not bound to any of the respiratory complexes. It moves freely in the inner mitochondrial membrane.
18. A part of Complex II catalyzes the conversion of succinate to fumarate in the citric acid cycle.
19. Three of the four respiratory complexes generate enough energy to phosphorylate ADP to ATP. Complex II is the sole exception.
20. Cytochrome *c* is not tightly bound to the mitochondrial membrane and can easily be lost in the course of cell fractionation. This protein is so similar in most aerobic organisms that cytochrome *c* from one source can easily be substituted for that from another source.
21. Succinate + $\frac{1}{2}O_2$ → Fumerate + H_2O
22. The components are in the proper orientation for the electrons to be transferred rapidly from one component to the next; if the components were in solution, speed would be limited to the rate of diffusion. A second advantage, which is actually a necessity, is that the components are properly positioned to facilitate the transport of protons from the matrix to the intermembrane space.
23. From an evolutionary standpoint, two different functions can be performed by identical structures or by structures that are close to identical, with only minor differences in the protein moieties. The organism saves a considerable amount of energy by not having to evolve—and to operate—two pathways.
24. The key point here is not the active site, which has a low tolerance for mutations, but the molecules with which the proteins in question are associated. Cytochromes are membrane-bound and must associate with other members of the electron transport chain; most mutations are likely to interfere with the close fit, and thus they are not preserved (because they are lethal). Globins, although soluble, still form some associations, so more mutations can be tolerated, with some limits. Hydrolytic enzymes are soluble and not likely to associate with other polypeptides except substrates. They can tolerate a higher proportion of mutations.
25. Having mobile electron carriers in addition to membrane-bound respiratory complexes allows electron transport to use the most readily available complex rather than to use the same one all the time.
26. The Q cycle allows for a smooth transition from two-electron carriers (NADH and $FADH_2$) to one-electron carriers (cytochromes).
27. The protein environment of the iron differs in each of the cytochromes, causing differences in the reduction potential.
28. All the reactions in the electron transport chain are electron-transfer reactions, but some of the reactants and products inherently transfer either one or two electrons, as the case may be.
29. The heme groups differ slightly in the various kinds of cytochromes. This is the main difference, with some modification due to the different protein environments.
30. Respiratory complexes contain a number of proteins, some of them quite large. This is the first difficulty. Like most proteins bound to membranes, the components of respiratory complexes are easily denatured on removal from their environment.

20.4 The Connection between Electron Transport and Phosphorylation

31. The F_1 portion of the mitochondrial ATP synthase, which projects into the matrix, is the site of ATP synthesis.
32. The F_0 portion of mitochondrial ATP synthase lies within the inner mitochondrial membrane, but the F_1 portion projects into the matrix.
33. The P/O ratio gives the number of moles of P_i consumed in the reaction ADP + P_i → ATP for each mole of oxygen atoms consumed in the reaction $\frac{1}{2}O_2 + 2H^+ \rightarrow 2H_2O$. It is a measure of the coupling of ATP production to electron transport.
34. The F_1 part of mitochondrial ATP synthase has a stationary domain (the $\alpha_3\beta_3\delta$ domain) and a domain that rotates (the $\gamma\varepsilon$ domain). This is exactly the arrangement needed for a motor.
35. A P/O ratio of 1.5 can be expected because oxidation of succinate passes electrons to coenzyme Q via a flavoprotein intermediate, bypassing the first respiratory complex.
36. Exact values for P/O ratios are difficult to determine because of the complexity of the systems that pump protons and phosphorylate ADP. The number of ADP molecules phosphorylated is directly related to the number of protons pumped across the membrane. This figure has been a matter of some controversy. It has been difficult for chemists and biochemists to accept uncertain stoichiometry.
37. The difficulties in determining the number of protons pumped across the inner mitochondrial membrane by respiratory complexes are those inherent in working with large assemblies of proteins that must be bound in a membrane environment to be active. As experimental methods improve, the task becomes less difficult.

20.5 The Mechanism of Coupling in Oxidative Phosphorylation

38. The chemiosmotic coupling mechanism is based on the difference in hydrogen ion concentration between the intermembrane space and the matrix of actively respiring mitochondria. The hydrogen ion gradient is created by the proton pumping that accompanies the transfer of electrons. The flow of hydrogen ions back into the matrix through a channel in the ATP synthase is directly coupled to the phosphorylation of ADP.
39. An intact mitochondrial membrane is necessary for compartmentalization, which in turn is necessary for proton pumping.
40. Uncouplers overcome the proton gradient on which oxidative phosphorylation depends.
41. In chemiosmotic coupling, the proton gradient is related to ATP production. The proton gradient leads to conformational changes in a number of proteins, releasing tightly bound ATP from the synthase as a result of the conformational change.
42. Dinitrophenol is an uncoupler of oxidative phosphorylation. The rationale was to dissipate energy as heat.
43. The energy released as protons pass through the F particles is actually used to cause conformational changes in the F_1 proteins, thereby releasing ATP. The "tight" conformation (one of three) provides a hydrophobic environment in which ADP is phosphorylated by adding P_i without requiring *immediate* energy.

20.6 Respiratory Inhibitors Can Be Used to Study Electron Transport

44. (a) Azide inhibits the transfer of electrons from cytochrome aa_3 to oxygen.
    (b) Antimycin A inhibits the transfer of electrons from cytochrome *b* to coenzyme Q in the Q cycle.
    (c) Amytal inhibits the transfer of electrons from NADH reductase to coenzyme Q.
    (d) Rotenone inhibits the transfer of electrons from NADH reductase to coenzyme Q.
    (e) Dinitrophenol is an uncoupler of oxidative phosphorylation.
    (f) Gramicidin A is an uncoupler of oxidative phosphorylation.
    (g) Carbon monoxide inhibits the transfer of electrons from cytochrome aa_3 to oxygen.

45. Methods exist to determine the amounts of the oxidized and reduced components of the electron transport chain present in a sample. If a respiratory inhibitor is added, the reduced form of the component before the blockage point in the chain accumulates as does the oxidized form of the component immediately after the blockage point.
46. Uncouplers overcome the proton gradient created by electron transport, whereas respiratory inhibitors block the flow of electrons.

20.7 Shuttle Mechanisms

47. The complete oxidation of glucose produces 30 molecules of ATP in muscle and brain and 32 ATP in liver, heart, and kidney. The underlying reason is the different shuttle mechanisms for transfer to mitochondria of electrons from the NADH produced in the cytosol by glycolysis.
48. The transport "product" (in the matrix) of the malate–aspartate shuttle is NADH, whereas that of the glycerol–phosphate shuttle is $FADH_2$. The latter shuttle can thus go *against* a transmembrane NADH concentration gradient, whereas the former cannot.

20.8 The ATP Yield from Complete Oxidation of Glucose

49. (a) 34
    (b) 32
    (c) 13.5
    (d) 17
    (e) 2.5
    (f) 12.5
50. The maximum yield of ATP, to the nearest whole number, is 3.

$$102.3 \text{ kJ released} \times \frac{1 \text{ ATP}}{30.5 \text{ kJ}} = 3.35 \text{ ATP}$$

One ATP is actually produced, so the efficiency of the process is

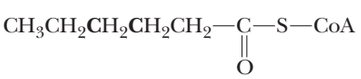
$$\frac{1 \text{ ATP}}{3 \text{ ATP}} \times 100 = 33.3\%$$

Chapter 21

21.1 Lipids Are Involved in the Generation and Storage of Energy

1. (a) For mobile organisms—such as a migrating hummingbird—weight can be a critical factor, and packing the most energy into the least weight is decidedly advantageous. A 2.5-g hummingbird needs to add about 2 g of fat for migration energy, which would increase body weight by 80%. The equivalent amount of energy stored as glycogen would be about 5 g, which would increase its body weight by 200%; the bird would never get off the ground!
   (b) For immobile plants, weight is not a critical factor, and it takes more energy to make fat or oil than it does to make starch. (The second law of thermodynamics would dictate that the energy obtained from oil would be less than that expended making oil. You can verify this numerically if you wish.) In the case of plant *seeds,* "compact" energy is beneficial, because the seed must be self-sufficient until enough growth has occurred to permit photosynthesis.

21.2 Catabolism of Lipids

2. Phospholipase A_1 hydrolyzes the ester bond to carbon-1 of the glycerol backbone; phospholipase A_2 hydrolyzes the ester bond to carbon-2 of the backbone.
3. A hormone signal activates adenylate cyclase, which makes cAMP. This activates protein kinases, which phosphorylate the lipases, thereby activating them.
4. Acyl-CoAs are high-energy compounds. An acyl-CoA has sufficient energy to initiate the β-oxidation process. The CoA is also a tag indicating that the molecule is destined for oxidation.
5. Acyl groups are esterified to carnitine to cross the inner mitochondrial membrane. There are transesterification reactions from the acyl-CoA to carnitine and from acylcarnitine to CoA (see Figure 21.5).
6. Acyl-CoA dehydrogenase removes hydrogens from adjacent carbons, creating a double bond and using FAD as coenzyme. β-Hydroxy-CoA dehydrogenase oxidizes an alcohol group to a ketone group and uses NAD^+ as a coenzyme.
7. 

   The two carbons shown in boldface type are the ones that will have the double bond between them. The orientation will be *trans.*
8. Seven carbon–carbon bonds are broken in the course of β-oxidation (see Figure 21.6).
9. In the liver, glycogen breakdown and gluconeogenesis would occur. In the muscle, glycogen breakdown and glycolysis would occur.

21.3 The Energy Yield from the Oxidation of Fatty Acids

10. One obtains 6.7 ATP per carbon and 0.42 ATP per gram for stearic acid versus 5 ATP per carbon and 0.17 ATP per gram for glucose. More energy is available from stearic acid than from glucose.
11. The processing of the acetyl-CoA through the citric acid cycle and the electron transport chain produces more energy than the processing of the NADH and $FADH_2$ produced during β-oxidation.
12. From seven cycles of β-oxidation: 8 acetyl-CoA, 7 $FADH_2$, and 7 NADH. From the processing of 8 acetyl-CoA in the citric acid cycle: 8 $FADH_2$, 24 NADH, and 8 GTP. From reoxidation of all $FADH_2$ and NADH: 22.5 ATP from 15 $FADH_2$, 77.5 ATP from 31 NADH. From 8 GTP: 8 ATP. Subtotal: 108 ATP. A 2-ATP equivalent was used in the activation step. Grand total: 106 ATP. The grand total for stearic acid was 120 ATP.
13. The humps of camels contain lipids that can be degraded as a source of metabolic water, rather than water as such.

21.4 Catabolism of Unsaturated Fatty Acids and Odd-Carbon Fatty Acids

14. For an odd-chain fatty acid, β-oxidation proceeds normally until the last round. When five carbons are left, that round of β-oxidation releases one acetyl-CoA and one propionyl-CoA. Propionyl-CoA cannot be further metabolized by β-oxidation; however, a separate set of enzymes converts propionyl-CoA into succinyl-CoA, which can then enter the citric acid cycle.
15. False. The oxidation of unsaturated fatty acids to acetyl-CoA requires a *cis–trans* isomerization and an epimerization, reactions that are not found in the oxidation of saturated fatty acids.
16. For a monounsaturated fatty acid, an additional enzyme is needed, the enoyl-CoA isomerase.
17. For a polyunsaturated fatty acid, two additional enzymes are needed, the enoyl-CoA isomerase and 2,4-dienoyl-CoA reductase.
18. From seven cycles of β-oxidation: 7 acetyl-CoA, 1 propionyl-CoA, 7 $FADH_2$, 7 NADH. From the processing of 7 acetyl-CoA in the citric acid cycle: 7 $FADH_2$, 21 NADH, and 7 GTP. From the processing of the propionyl-CoA: –1 ATP for conversion to succinyl-CoA, –1 GTP from the citric acid cycle, and 1 NADH and 1 $FADH_2$ from the citric acid cycle. From reoxidation of all $FADH_2$ and NADH: 22.5 ATP from 15 $FADH_2$, and 72.5 ATP from 29 NADH. From 8 GTP: 8 ATP. Subtotal: 103 ATP. Subtract a 2-ATP equivalent used in activation step and a 1-ATP equivalent used in the conversion to succinyl-CoA for a grand total of 100 ATP.
19. An 18-carbon saturated fatty acid yields 120 ATP. For a monounsaturated fatty acid, the double bond eliminates the step that produces $FADH_2$, so there would be 1.5 ATP less for oleic acid, or 118.5 ATP total.
20. An 18-carbon saturated fatty acid yields 120 ATP. For a diunsaturated fatty acid with the bonds in the Δ^9 and Δ^{12} positions, the first double bond eliminates an $FADH_2$. The second double bond uses an NADPH, which we are guessing is the same cost as using an NADH. Thus a total of 4 ATP are lost, compared with a saturated fatty acid, so the total is 116 ATP.
21. It would take seven cycles of β-oxidation to release 14 carbons as acetyl-CoA, with the last three being released as propionyl-CoA.
22. Fats cannot produce a net yield of glucose because they must enter the citric acid cycle as the two-carbon unit acetyl-CoA. In the first few steps, two carbons are released as CO_2. However, an odd-chain fatty acid can be considered partially glucogenic because the final three carbons become succinyl-CoA and enter the citric acid cycle after the decarboxylation steps. Thus, if an extra succinyl-CoA is added, it can then be drawn off later as malate and used for gluconeogenesis without removing the steady-state level of citric acid cycle intermediates.

21.5 Ketone Bodies

23. Ketones are produced when there is an imbalance in lipid catabolism, compared with carbohydrate catabolism. If fatty acids are being β-oxidized to produce acetyl-CoA, but there is insufficient oxaloacetate because it is being drawn off for gluconeogenesis, the acetyl-CoA molecules combine to form ketone bodies.
24. Two acetyl-CoA molecules combine to form acetoacetyl-CoA. This can then release coenzyme A to yield acetoacetate, which can be converted either to β-hydroxybutyrate or to acetone.
25. If the reason for passing out is uncontrolled diabetes, the doctor expects to smell acetone on the breath, since the otherwise unused sugars are being converted to fats and ketone bodies.

26. Ethanol is converted to acetaldehyde and then to acetic acid. Humans can use that acetic acid only for energy, or they can convert it to fatty acids and other lipids.
27. The metallic taste may be due to acetone, which means that your friend may have a mild state of ketosis. Ask if your friend has consulted a doctor about the diet regimen, and perhaps recommend either backing off from such a low-calorie diet or drinking more water to flush the system more thoroughly.

21.6 Fatty-Acid Biosynthesis

28. The two pathways have in common the involvement of acetyl-CoA and thioesters, and each round of breakdown or synthesis involves two-carbon units. The differences are many: malonyl-CoA is involved in biosynthesis, not in breakdown; thioesters involve CoA in breakdown and involve acyl carrier proteins in biosynthesis; biosynthesis occurs in the cytosol, but breakdown occurs in the mitochondrial matrix; breakdown is an oxidative process that requires NAD^+ and FAD and produces ATP by electron transport and oxidative phosphorylation, whereas biosynthesis is a reductive process that requires NADPH and ATP.
29. Step 1: biotin is carboxylated using bicarbonate ion (HCO_3^-) as the source of the carboxyl group. Step 2: the carboxylated biotin is brought into proximity with enzyme-bound acetyl-CoA by a biotin carrier protein. Step 3: the carboxyl group is transferred to acetyl-CoA, forming malonyl-CoA.
30. It is a molecule that commits itself to fatty-acid synthesis. It is also a potent inhibitor of carnitine acyltransferase I, thereby shutting down β-oxidation.
31. ACP, citrate, cytosol, *trans* double bonds, D-alcohols, β-reduction, NADPH, malonyl-CoA (except for one acetyl-CoA), and a multifunctional enzyme complex.
32. In β-oxidation, FAD is the coenzyme for the first oxidation reaction, while NAD^+ is the coenzyme for the second. In fatty-acid synthesis, NADPH is the coenzyme for both. The β-hydroxy-acyl group in β-oxidation has the L-configuration, while it has the D-configuration in fatty acid synthesis.
33. Both have a phosphopantetheine group at the active end. In coenzyme A, this group is attached to 2′-phospho-AMP; in ACP, it is attached to a serine residue of a protein.
34. ACP is a molecule that earmarks acyl groups for fatty-acid synthesis. It can be managed separately from acyl-CoA groups. Also, the ACP attaches to the acyl groups like a "swinging arm" that tethers it to the fatty-acid synthase complex.
35. Linoleate and linolenate cannot be synthesized by the body and must therefore be obtained from dietary sources. Mammals cannot produce a double bond beyond carbon atom 9 of fatty acids.
36. Acyl-CoA intermediates are essential in the conversion of fatty acids to other lipids.
37. Acetyl groups condense with oxaloacetate to form citrate, which can cross the mitochondrial membrane. Acetyl groups are regenerated in the cytosol by the reverse reaction.
38. If acetyl-carnitine forms in the matrix of the mitochondrion, it can be translocated to the cytosol via the carnitine translocase. Thus, this could represent another way of shuttling acetyl units out of the mitochondria for synthesis.
39. Energy is needed to condense an acetyl group to the growing fatty acid. In theory, such could be done with acetyl-CoA, using ATP. In practice, the ATP is used to convert acetyl-CoA to malonyl-CoA; the condensation of the acetyl moiety of malonyl-CoA is driven in part by the accompanying decarboxylation and requires no additional energy. A possible reason for this is to avoid a metabolic confusion of pathways, perhaps particularly important in (uncompartmented) prokaryotes; one could envision an acetyl-CoA from degradation being used immediately for synthesis. Malonyl-CoA says "synthesis"; acetyl-CoA says "degradation."
40. (a) The lipoate "swinging arm" of the pyruvate dehydrogenase complex.
    (b) The "arm" or ACP carries the group to be acted on from one enzyme to another (avoiding a diffusion-limited process and also positioning key groups correctly). In the case of the ACP, the group to be acted on (β-carbon) is always the same distance from the ACP, regardless of the length of the growing fatty acid, and thus the critical group is always in proximity to the active sites of the several pertinent enzymes.

21.7 Synthesis of Acylglycerols and Compound Lipids

41. The glycerol comes from degradation of other acylglycerols or from glycerol-3-phosphate derived from glycolysis.
42. The activating group found on the acylglycerol is cytidine diphosphate.
43. In prokaryotes, CTP reacts with phosphatidic acid to give a CDP-diacylglycerol. This reacts with serine to give phosphatidylserine, which decarboxylates to phosphatidylethanolamine. In eukaryotes, CDP-ethanolamine reacts with a diacylglycerol to give phosphatidylethanolamine.

21.8 Cholesterol Biosynthesis

44. In steroid biosynthesis, three acetyl-CoA molecules condense to form the six-carbon mevalonate, which then gives rise to a five-carbon isoprenoid unit. A second and then a third isoprenoid unit condense, giving rise to a 10-carbon and then a 15-carbon unit. Two of the 15-carbon units condense, forming a 30-carbon precursor of cholesterol.
45. See Figure 21.24.
46. Bile acids and steroid hormones.
47. All steroids have a characteristic fused-ring structure, implying a common biosynthetic origin.
48. One oxygen atom from O_2 is needed to form the epoxide. The NADPH is needed to reduce the other oxygen atom to water.
49. Cholesterol is nonpolar and cannot dissolve in blood, which is an aqueous medium.
50. Bile salts are made from cholesterol, and cholesterol is taken from the body into the intestine in the bile fluid.

Chapter 22

22.1 Chloroplasts Are the Site of Photosynthesis

1. In the fall, the chlorophyll in leaves is lost, and the red and yellow colors of the accessory pigments become visible, accounting for fall foliage colors.
2. The bean sprouts are grown in the dark to prevent them from turning green; most customers will not purchase green sprouts.
3. Iron and manganese in chloroplasts; iron and copper in mitochondria. Note that all these are transition metals, which can easily undergo redox reactions.
4. Both chloroplasts and mitochondria have an inner and outer membrane. Both have their own DNA and ribosomes. Chloroplasts, however, have a third membrane, the thylakoid membrane.
5. Chlorophyll has a cyclopentanone ring fused to the tetrapyrrole ring, a feature that does not exist in heme. Chlorophyll contains magnesium, whereas heme contains iron. Chlorophyll has a long side chain based on isoprenoid units, which is not found in heme.
6. Only a relatively small portion of the visible spectrum is absorbed by chlorophylls. The accessory pigments absorb light at additional wavelengths. As a result, most of the visible spectrum can be harnessed in light-dependent reactions.
7. It is one more piece of evidence that is consistent with the evolution of chloroplasts from independent bacterial organisms.

22.2 Photosystems I and II and the Light Reactions of Photosynthesis

8. By and large, the synthesis of NADPH in chloroplasts is the reverse of NADH oxidation in mitochondria. The net electron flow in chloroplasts is the reverse of that in the mitochondria, although different carriers are involved.
9. When light impinges on the reaction center of *Rhodopseudomonas,* the special pair of chlorophylls there is raised to an excited energy level. An electron is passed from the special pair to accessory pigments, first pheophytin, then menaquinone, and finally to ubiquinone. The electron lost by the special pair of chlorophylls is replaced by a soluble cytochrome, which diffuses away. The separation of charge represents stored energy (see Figure 21.9).
10. In photosystem I and in photosystem II, light energy is needed to raise the reaction-center chlorophylls to a higher energy level. Energy is needed to generate strong enough reducing agents to pass electrons to the next of the series of components in the pathway.
11. No. Most chlorophylls are light-harvesting molecules that transfer energy to the special pair that takes part in the light reactions.
12. The electron transport chain in chloroplasts, like that in mitochondria, consists of proteins, such as plastocyanin, and protein complexes, such as the cytochrome b_6–f complex. It also contains mobile electron carriers, such as pheophytin and plastoquinone (equivalent to coenzyme Q), which is also true of the mitochondrial electron transport chain.
13. Probably the electron transport chain in chloroplasts. Chloroplasts generate molecular oxygen; mitochondria use it. The early atmosphere almost certainly lacked molecular oxygen. Only when photosynthesis introduced oxygen into the atmosphere would oxygen be needed.

14. Electron transport and ATP production are coupled to each other by the same mechanism in mitochondria and chloroplasts. In both cases, the coupling depends on the generation of a proton gradient across the inner mitochondrial membrane or across the thylakoid membrane, as the case may be.
15. In mitochondria, both a proton gradient (chemical) and an electrochemical gradient (based on charge) are formed, both contributing to the total potential energy. In chloroplasts, only a proton gradient is formed, because ions move across the thylakoid membrane and neutralize charge. The proton gradient alone is considerably less efficient.
16. With very few exceptions, life directly or indirectly depends on photosynthesis. The electric current is the flow of electrons from water to $NADP^+$, a light-requiring process. The "current" continues in the light-independent reactions, with electrons flowing from NADPH to *bis*phosphoglycerate, which ultimately yields glucose.
17. Photosystem II requires more energy than photosystem I. The shorter wavelength of light means a higher frequency. Frequency, in turn, is directly proportional to energy.
18. It is quite reasonable to list reduction potentials for the electron-transfer reactions of photosynthesis. They are entirely analogous to the electron-transfer reactions in mitochondria, for which we listed standard reduction potentials in Chapter 20.
19. A photosynthetic reaction center is analogous to a battery because its reactions produce a charge separation. The charge separation is comparable to the stored energy of the battery.
20. The electron transport chains of mitochondria and chloroplasts are similar. In mitochondria, antimycin A inhibits electron transfer from cytochrome *b* to coenzyme Q in the Q cycle. By analogy, it can be argued that antimycin A inhibits electron flow from plastoquinone to cytochrome b_6–*f*. A Q cycle may also operate in chloroplasts.
21. Oxygen produced in photosynthesis comes from water. The oxygen-evolving complex is part of the series of electron-transfer reactions from water to NADPH. Carbon dioxide is involved in the dark reactions, which are different reactions that take place in another part of the chloroplast.
22. It is well established that the path of electrons in photosynthesis goes from photosystem II to photosystem I. The reason for the nomenclature is that photosystem I is easier to isolate than photosystem II and was studied more extensively at an earlier date.
23. It would take much work to establish the number of protons pumped across the thylakoid membrane. This is partly the result of experience with mitochondria and partly a prediction based on the greater complexity of structure in the chloroplast.
24. The oxygen-evolving complex of photosystem II passes through a series of five oxidation states (designated as S_0 through S_4) in the transfer of four electrons in the process of evolving oxygen (Figure 22.6). One electron is passed from water to photosystem II for each quantum of light. In the process, the components of the reaction center go successively through oxidation states S_1 through S_4. The S_4 decays spontaneously to the S_0 state and, in the process, oxidizes two water molecules to one oxygen molecule. Four protons are released simultaneously.
25. When the loosely bound cytochrome diffuses away, a charge separation is induced. This separation of charge represents stored energy.
26. The similarity of ATP synthase in chloroplasts and mitochondria supports the idea that both may have arisen from free-living bacteria.

22.3 Photosynthesis and ATP Production

27. In cyclic photophosphorylation, the excited chlorophyll of photosystem I passes electrons directly to the electron transport chain that normally links photosystem II to photosystem I. This electron transport chain is coupled to ATP production (see Figure 22.8).
28. Both depend on a proton gradient, resulting from the flow of electrons. In chloroplasts, protons come from the splitting of water to produce oxygen. In mitochondria, protons come from the oxidation of NADH and ultimately consume oxygen and produce water.
29. The proton gradient is created by the operation of the electron transport chain that links the two photosystems in noncyclic photophosphorylation.
30. ATP can be produced by chloroplasts in the absence of light if some way exists to form a proton gradient.
31. Cyclic photophosphorylation can take place when the plant needs ATP but does not have a great need for NADPH. Noncyclic photophosphorylation can take place when the plant needs both.

22.4 Evolutionary Implications of Photosynthesis with and without Oxygen

32. Many electron donors other than water are possible in photosynthesis. This is especially the case in bacteria, whose photosystems do not have strong enough oxidizing agents to oxidize water. Some of the alternative electron donors are H_2S and organic compounds.
33. A prokaryotic organism that contains both chlorophyll *a* and chlorophyll *b* could be a relic of an evolutionary way station in the development of chloroplasts.

22.5 Dark Reactions of Photosynthesis Fix CO_2

34. Rubisco is the principal protein in chloroplasts in all green plants. This wide distribution makes it likely to be the most abundant protein in nature.
35. The amino acid sequence of the catalytic subunits of rubisco is encoded by chloroplast genes, whereas that of the regulatory subunits is encoded by nuclear genes.
36. Gluconeogenesis and the pentose phosphate pathway have a number of reactions similar to those of the dark reactions of photosynthesis.
37. From the standpoint of thermodynamics, the production of sugars in photosynthesis is the reverse of the complete oxidation of a sugar such as glucose to CO_2 and water. The complete oxidation reaction produces six moles of CO_2 for each mole of glucose oxidized. To get the energy change for the fixation of one mole of CO_2, change the sign of the energy for the complete oxidation of glucose and divide by 6.
38. Glucose synthesized by photosynthesis is not uniformly labeled because only one molecule of CO_2 is incorporated into each molecule of ribulose-1,5-*bis*phosphate, which then goes on to give rise to sugars.
39. If rubisco was one of the first protein enzymes to arise early in the evolution of life, it may not have the efficiency of protein enzymes that evolved later, when evolution was more dependent on modifying and adapting existing proteins.
40. Their DNA is circular. Their ribosomes are more like those of bacteria than those of eukaryotes. Their aminoacyl-tRNA synthetases use bacterial tRNAs but not eukaryotic tRNAs. In general, they do not have introns in their genomes. Their mRNA uses a Shine-Dalgarno sequence.
41. The pathway borrows heavily from the nonoxidative branch of the pentose phosphate pathway and from gluconeogenesis. Without doubt, the pathways yield sugars as well as NADPH for reductive biosynthesis. Thus, only a few new enzymes would have to evolve through mutations to enable the complete Calvin cycle to function.
42. Atmospheric oxygen is a consequence of photosynthesis. Rubisco evolved before there was a significant amount of oxygen in the atmosphere.
43. The condensation of ribulose-1,5-*bis*phosphate with carbon dioxide to form two molecules of 3-phosphoglycerate is the actual carbon dioxide fixation. The rest of the Calvin cycle regenerates ribulose-1,5-*bis*phosphate.
44. Organisms would need only a few mutations giving rise to the enzymes unique to the Calvin cycle. The rest of the pathway is already in place.
45. Six molecules of carbon dioxide fixed in the Calvin cycle do not end up in the same glucose molecule. However, labeling experiments show that six carbon atoms are incorporated into sugars for every six carbon dioxide molecules that enter the Calvin cycle.

22.6 CO_2 Fixation in Tropical Plants

46. In tropical plants, the C_4 pathway is operative in addition to the Calvin cycle.
47. In C_4 plants, when CO_2 enters the leaf through pores in the outer cells, it reacts first with phosphoenolpyruvate to produce oxaloacetate and P_i in the mesophyll cells of the leaf. Oxaloacetate is reduced to malate, with the concomitant oxidation of NADPH. Malate is then transported to the bundle-sheath cells (the next layer) through channels that connect two kinds of cells. These reactions do not take place in C_3 plants.
48. Photorespiration is a pathway in which glycolate is a substrate oxidized by rubisco acting as an oxygenase, rather than as a carboxylase. Photorespiration is not completely understood.
49. Three reasons come to mind. (1) Light energy is usually not limiting. (2) The plants have small pores to prevent water loss, but this also limits CO_2 uptake. (3) The C_4 pathway allows for increasing the CO_2 concentration in the inner chloroplast, which would not be otherwise possible with the small pores.
50. Most plants would be more productive in the absence of photorespiration. There is another side to this picture, however. The oxygenase activity appears to be an unavoidable, wasteful activity of rubisco. Photorespiration is a salvage pathway that saves some of the carbon that would be lost due to the oxygenase activity of rubisco. Photorespiration is essential to plants even though the plant pays the price in loss of ATP and reducing power; mutations that affect this pathway can be lethal.

Chapter 23

23.1 Nitrogen Metabolism: An Overview

1. Nitrogen-fixing bacteria (symbiotic organisms that form nodules on the roots of leguminous plants, such as beans and alfalfa) and some free-living microbes and cyanobacteria can fix nitrogen. Plants and animals cannot.

23.2 Nitrogen Fixation

2. Nitrogen is fixed by the nitrogenase reaction, in which N_2 is converted to NH_4^+. Very few organisms have this enzyme, which can catalyze the breaking of the triple bond in molecular nitrogen. The glutamate dehydrogenase reaction and the glutamine synthase reactions assimilate nitrogen:

$$NH_4^+ + \alpha\text{-Ketoglutarate} \rightleftharpoons \text{Glutamate} + \text{Water (NADPH required)}$$
$$NH_4^+ + \text{Glutamate} \rightleftharpoons \text{Glutamine (ATP required)}$$

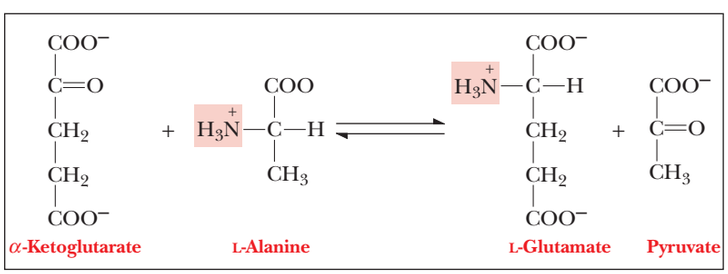
3. The chemical synthesis of ammonia from H_2 and N_2.
4. $N_2 + 8e^- + 16ATP + 10H^+ \rightarrow 2NH_4^+ + 16ADP + 16P_i + H_2$ is the half reaction for reduction via nitrogenase. The oxidation reaction varies with species.
5. The nitrogenase complex is made up of ferredoxin, dinitrogenase reductase, and nitrogenase. Dinitrogenase reductase is an iron–sulfur protein, whereas nitrogenase is an iron–molybdenum protein. The Fe—S protein is a dimer ("the iron butterfly"), with the iron–sulfur cluster located at the butterfly's head. The nitrogenase is even more complicated, with several types of subunits arranged into tetramers.

23.3 Feedback Inhibition in Nitrogen Metabolism

6. Pathways that use nitrogen to make amino acids, purines, and pyrimidines are controlled by feedback inhibition. The final product, such as CTP, inhibits the first or an early step in its synthesis.
7. Feedback control mechanisms slow down long biosynthetic pathways at or near their beginnings, saving energy for the organism.
8. Because all the components of a cycle are regenerated, only small amounts ("catalytic quantities") are needed. This is important from an energy standpoint and, perhaps with some compounds, because of insolubility problems.

23.4 Amino Acid Biosynthesis

9. They are all interrelated. α-Ketoglutarate can be changed to glutamate via transamination or glutamate dehydrogenase. Glutamine synthetase makes glutamine out of glutamate.
10.



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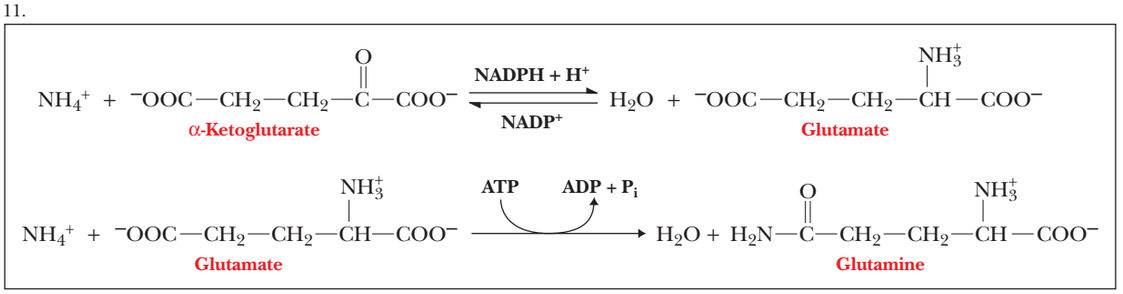

12. Glutamine synthetase catalyzes the following reaction and uses energy: $NH_4^+ + \text{Glutamate} + ATP \rightarrow \text{Glutamine} + ADP + P_i + H_2O$. Glutaminase catalyzes the following reaction and does not use energy directly: $\text{Glutamine} + H_2O \rightarrow \text{Glutamate} + NH_4^+$.
13. See Figure 23.8.
14. The principal ones are tetrahydrofolate and *S*-adenosylmethionine.
15. See Figure 23.11.
16. Conversion of homocysteine to methionine using *S*-adenosylmethionine as the methyl donor gives no net gain; one methionine is needed to produce another methionine.
17. Glutamate + α-Keto acid → α-Ketoglutarate + Amino acid
18. See the *S*-adenosylmethionine structure in Figure 23.15. The reactive methyl group is indicated.
19. Sulfanilamide inhibits folic acid biosynthesis.
20. Methionine can play a dual role. In addition to providing a hydrophobic group, methionine (in the form of *S*-adenosylmethionine) can act as a methyl group donor.

23.5 Essential Amino Acids

21. The essential amino acids are those with branched chains, aromatic rings, or basic side chains.
22. In both cases, the requirements are those given in Table 23.1.

23.6 Amino Acid Catabolism

23. Five α-amino acids are involved directly in the urea cycle (ornithine, citrulline, aspartate, arginosuccinate, and arginine). Of those, only aspartate and arginine are also found in proteins.
24. $H^+ + HCO_3^- + 2NH_3 + 3ATP \rightarrow NH_2CONH_2 + 2ADP + 2P_i + AMP + PP_i + 2H_2O$. The urea cycle is linked to the citric acid cycle by fumarate and by aspartate, which can be converted to malate by transamination (see Figure 23.19).
25. Ornithine is similar to lysine, but it has one fewer methylene group in the side chain. Citrulline is a keto version of arginine with a side chain C═NH_2^+ replaced by C═O.
26. Aspartate and arginosuccinate are the amino acids that link the two pathways. Aspartate is made by transamination of OAA. The aspartate then combines with citrulline to form arginosuccinate, which then releases a fumarate to go back to the TCA cycle.
27. Each round of the urea cycle costs 4 ATP, two to make carbamoyl-phosphate and effectively two (ATP → AMP) to make arginosuccinate.

28. It is controlled by a special effector molecule, *N*-acetylglutamate, which is itself controlled by levels of arginine.
29. When arginine levels build up, it means that the urea cycle is going too slow and not enough carbamoyl-phosphate is available to react with ornithine.
30. Glutamate brings ammonia groups to the matrix of the mitochondria for the urea cycle. High levels of glutamate stimulate the urea cycle.
31. Glucogenic amino acids are degraded to pyruvate or one of the citric acid cycle intermediates found after the decarboxylation steps, such as succinate or malate. Ketogenic amino acids are degraded to acetyl-CoA or acetoacetyl-CoA.
32. (a) Glucogenic
    (b) Glucogenic
    (c) Glucogenic
    (d) Ketogenic
    (e) Glucogenic
    (f) Ketogenic
33. Fish excrete excess nitrogen as ammonia, and birds excrete it as uric acid. Mammals excrete it as urea.
34. Because ostriches don't fly, one could argue that they would excrete their excess nitrogen as urea. On the other hand, they are birds, and as such probably have the same metabolism of their lighter counterparts, and might likely excrete it as uric acid.
35. The amounts of arginine necessary in the urea cycle are only catalytic. If arginine from the cycle is used for protein synthesis, the cycle becomes depleted.
36. A high-protein diet leads to increased production of urea. Drinking more water increases the volume of urine, ensuring elimination of the urea from the body with less strain on the kidneys than if urea were at a higher concentration.
37. The metabolism of amino acids encourages urine formation and actually a greater thirst and need for water.
38. Several enzymes, resulting from mutations, are needed for the urea cycle. Most mutations tend to be lost unless they provide some survival value. It seems improbable that all the mutations needed for all the enzymes of the cycle would arise nearly simultaneously. However, the origin of the cycle can be rather easily explained on the premise that only one new enzyme (arginase) was needed. The other enzymes of the cycle are needed for the biosynthesis of arginine. As a component of proteins, arginine was presumably needed before there was a need for a urea cycle. This is an example of nature using features already available to bring about a new function.

23.7 Purine Biosynthesis

39. Since folic acid is critical to the formation of purines, antagonists of folic acid metabolism are used as chemotherapy drugs to inhibit nucleic acid synthesis and cell growth. Rapidly dividing cells, such as those found in cancer and tumors, are more susceptible to these antagonists.
40. All four nitrogen atoms of the purine ring are derived from amino acids: two from glutamine, one from aspartate, and one from glycine. Two of the five carbon atoms (adjacent to the glycine nitrogen) also come from glycine, two more come from tetrahydrofolate derivatives, and the fifth comes from CO_2.
41. In inosine, carbon-6 of the ring is a ketone group; in adenosine, carbon-6 is bound to an amino group.
42. Tetrahydrofolate is a carrier of carbon groups. Two of the carbons in the purine ring are donated by tetrahydrofolate.
43. The conversion of IMP to GMP produces one NADH and uses the equivalent of 2 ATP because an ATP is converted to AMP. Because NADH gives rise to 2.5 ATP if it goes into the electron transport chain, we can say that the conversion results in a net production of ATP.
44. There is a complicated system of feedback inhibition for the production of purine-containing nucleotides. The final products, ATP and GTP, feed back to inhibit the first steps starting from ribose-5-phosphate. In addition, each intermediate, such as AMP or ADP, can also inhibit the first step. Also, each of the three forms for each nucleotide inhibit the committed reaction from IMP that eventually decides which purine nucleotide is made.

23.8 Purine Catabolism

45. The purine salvage reaction that produces GMP requires the equivalent of 2 ATP. The pathway to IMP and then to GMP requires the equivalent of 8 ATP.
46. In most mammals, uric acid is converted to allantoic acid, which is much more water soluble than uric acid.

23.9 Pyrimidine Biosynthesis and Catabolism

47. In purine nucleotide biosynthesis, the growing purine ring is covalently bonded to ribose; the ribose is added after the ring is synthesized in pyrimidine nucleotide biosynthesis.
48. Purines break down to various products, depending on the species. These products are then excreted, representing a major means of nitrogen excretion for many organisms. Pyrimidine catabolism yields, in addition to NH_4^+ and CO_2, the salvageable product β-alanine, which is a breakdown product of both cytosine and uracil.

23.10 Conversion of Ribonucleotides to Deoxyribonucleotides

49. Both thioredoxin and thioredoxin reductase are proteins involved in the conversion of ribonucleotides to deoxyribonucleotides. Thioredoxin is an intermediate carrier of electrons and hydrogens, and thioredoxin reductase is the enzyme that catalyzes the process.

23.11 Conversion of dUTP to dTTP

50. Fluorouracil substitutes for thymine in DNA synthesis. In rapidly dividing cells, such as cancer cells, the result is the production of defective DNA, causing cell death.
51. The DNA of fast-growing cells, such as those of the hair follicles, is damaged by chemotherapeutic agents.

Chapter 24

24.1 Connections between Metabolic Pathways

1. ATP and NADPH are the two molecules that link the most pathways.
2. Acetyl-CoA, pyruvate, PEP, α-ketoglutarate, succinyl-CoA, oxaloacetate, and several sugar phosphates, such as glucose-6-phosphate and fructose-6-phosphate.
3. (a) Fructose-6-phosphate—from the pentose phosphate pathway (PPP).
   (b) Oxaloacetate—to phosphoenolpyruvate in gluconeogenesis, to and from aspartate, to the glyoxylate cycle via citrate.
   (c) Glucose-6-phosphate—to PPP, to and from glycogen in animals, to starch in plants.
   (d) Acetyl-CoA—to and from fatty acids, to steroids (and isoprenoids), some amino acid degradations, to the glyoxylate cycle via citrate.
   (e) Glyceraldehyde-3-phosphate—to reverse PPP.
   (f) α-Ketoglutarate—to and from glutamate.
   (g) Dihydroxyacetone phosphate—to and from the glycerol moiety of triacylglycerols and phosphoacylglycerols.
   (h) Succinyl-CoA—degradation of fatty acids with odd numbers of carbon atoms, some amino acid degradation.
   (i) 3-Phosphoglycerate—appears in the Calvin cycle.
   (j) Fumarate—some amino acid degradations.
   (k) Phosphoenolpyruvate—from oxaloacetate in gluconeogenesis.
   (l) Citrate—to the glyoxylate cycle, transport across the mitochondrial membrane for fatty acid and steroid synthesis.
   (m) Pyruvate—fermentation, to gluconeogenesis, also to and from alanine.
4. When the body breaks down proteins to supply material for gluconeogenesis, the increased urea output results in greater urine production, which uses water stored in the body. Fat metabolism also produces much metabolic water.

5. (a) High ATP or NADH concentration and the citric acid cycle: isocitrate dehydrogenase (and the citric acid cycle) would be inhibited. The resulting pileup of acetyl-CoA (or citrate) would stimulate fatty acid and steroid synthesis, gluconeogenesis, and (in plants and some microorganisms) the glyoxylate cycle.
   (b) High ATP concentration and glycolysis: phosphofructokinase-1 (and glycolysis) would be inhibited. Glucose-6-phosphate would pile up, stimulating glycogen (or starch) synthesis, the oxidative pentose phosphate pathway, or glucose formation. (c) High NADPH concentration and the pentose phosphate pathway: the oxidative branch of the pentose phosphate pathway would be inhibited, thus making glucose-6-phosphate available for other purposes. These include glycolysis, glycogen synthesis, glucose synthesis, and the "reverse" pentose phosphate pathway (yielding only pentose phosphate). (d) High fructose-2,6-*bis*phosphate concentration and gluconeogenesis: fructose-2,6-*bis*phosphate inhibits fructose-1,6-*bis*phosphatase and activates phosphofructokinase-1. Gluconeogenesis would thus be inhibited and glycolysis would be stimulated, as would the reverse pentose phosphate pathway and the production of glycerol phosphate for lipids.
6. Many compounds, such as oxaloacetate, pyruvate, and acetyl-CoA, play a role in a number of reactions. More to the point, the end products of some pathways are the starting points of others. Each pathway is one aspect of an overall metabolic scheme.
7. The *effect* of biochemical pathways can be reversed. Examples include glycolysis and gluconeogenesis, glycogen formation and synthesis, and the pentose phosphate pathway. The details are not completely reversible. An irreversible step in one pathway tends to be replaced with another reaction, catalyzed by another enzyme.
8. Transport processes are especially important for substances, such as oxaloacetate, that cannot cross the mitochondrial membrane. The same is true for electrons. Shuttle mechanisms must exist to transport electrons as the reduced form of important compounds. Compounds that cannot cross the membrane must be converted to ones that can, and then must be converted back to their original form on the other side of the membrane.
9. When a pathway has a number of steps, it is possible for energy changes to take place in steps of manageable size. It also allows for control of a pathway to be exercised at more points than would be the case if there were only a few steps.
10. The possibilities are limitless. Even more to the point, some discovery that no one expects can open even more possibilities.

24.2 Biochemistry and Nutrition

11. The old pyramid assumed that all carbohydrates and fats were the same and that carbohydrates were good and all fats were bad. The new pyramid recognizes that not all carbohydrates are good and not all fats are bad. Complex carbohydrates are placed lower down on the new pyramid, whereas processed ones are placed higher. Essential fats and oils are included as necessary food types. Also, dairy consumption recommendations have been reduced.
12. Fats and carbohydrates can be stored when they are consumed in excess. Fats are stored as triacylglycerols and carbohydrates are stored as glycogen. However, proteins consumed in excess are not stored. The extra protein is broken down. The amino groups are released as urea and the carbon skeletons are stored as carbohydrate or fat.
13. Saturated fatty acids have been correlated with increased levels of LDL, which have been shown to be an indicator of high risk for heart disease.
14. Leptin is a hormone that affects metabolism. It affects the brain to suppress appetite and it affects metabolism directly by stimulating fatty-acid oxidation and inhibiting fatty-acid synthesis.
15. Yes, cholecalciferol is made in the body, and many of its functions are hormone-like in nature.
16. Carbohydrates are the main energy source. Excess fat consumption can lead to the formation of "ketone bodies" and to atherosclerosis. Diets extremely high in protein can put a strain on the kidneys.
17. The liver is the primary organ for alcohol metabolism and for disposing of drugs (legal, illegal, and accidental) and halocarbon compounds. When the liver spends its time dealing with these other tasks, it may not be able to carry out its other normal functions; in essence, prolonged exposure to any such "toxin" overworks the liver.
18. Vitamin A is a lipid-soluble vitamin, which can accumulate in the body. Overdoses of this vitamin can be toxic.
19. Low levels of iodine in the diet often lead to hypothyroidism and an enlarged thyroid gland (goiter). This condition has largely been eliminated by the addition of sodium iodide to commercial table salt.
20. Lucullus breaks down the protein in the tuna to amino acids, which in turn undergo the urea cycle and the breakdown of the carbon skeleton described in Chapter 23, eventually leading to the citric acid cycle and electron transport. In addition to protein catabolism, Griselda breaks down the carbohydrates to sugars, which then undergo glycolysis and enter the citric acid cycle. (Gratuitous information: Lucullus was a notorious Roman gourmand. In medieval literature, Griselda was the name usually given to a forbearing, long-suffering woman.)
21. All amino acids must be present at the same time for protein synthesis to occur. Newly synthesized proteins are necessary for growth in the immature rats.
22. The weight loss is due to correction of the bloating caused by retention of liquids.
23. After a person is fully grown, many amino acids are scavenged and recycled by the body. Because all proteins contain at least some of these two amino acids, there are enough to maintain the body. It should be noted that both again become essential if there is disease or tissue damage and that arginine is required for sperm production in males.
24. The early colonists always cooked in iron pots; enough iron is leached out to supply required amounts, as long as the body is able to absorb it. (Glass cookware did not become available until after World War I, and aluminum cookware was not available until after World War II.)
25. Diets high in fiber are usually lower in fats, especially saturated fats; fiber adsorbs many potentially toxic substances, such as cholesterol and halocarbons, preventing their absorption into the body; fiber decreases transit time through the intestine, so any toxic materials in food remain in the body for less time and have a smaller chance of being absorbed or otherwise causing problems.
26. This claim has a chemical basis. Calcium carbonate dissolves in stomach acid, releasing calcium ion in its usual hydrated form. Calcium citrate is likely to have the calcium ion bound to the citrate in a manner similar to iron in heme. Consequently, the charge of the calcium ion is effectively decreased. Calcium bound to citrate can pass a cell membrane more easily than a hydrated calcium ion.
27. Alcohol provides calories but does not provide vitamins. This is one of the leading causes of malnutrition. Metabolizing alcohol involves an enzyme (alcohol dehydrogenase) with thiamine pyrophosphate (TPP) as a cofactor. The cofactor, in turn, is a metabolite of vitamin B_1, leading to severe deficiencies.
28. Metal ions play a role in the structure and function of proteins and some coenzymes. They tend to do so because they operate as Lewis acids.
29. Severe depletion of glycogen often results in a rebound effect, in which so much is made that some is stored in inappropriate tissues, including the heart, and mineral imbalances often occur. It is best to exercise moderately before the glycogen loading because then the glycogen is stored more effectively and safely in the liver and muscle tissue where it is most needed.
30. Nutrients and water turn over in the body, sometimes very frequently. This implies that an organism is an open system. Equilibrium requires a closed system. Consequently, an organism can reach a steady state, but never equilibrium.

24.3 Hormones and Second Messengers

31. Hormones can have several different kinds of chemical structures, including steroids, polypeptides, and amino acid derivatives.
32. The anterior pituitary stimulates release of trophic hormones, which in turn stimulate specific endocrine glands; the workings of the adrenal cortex, the thyroid, and the gonads can all be affected as a result. The adrenal cortex produces adrenocortical hormones, including glucocorticoids (involved in carbohydrate metabolism, inflammatory reactions, and reaction to stress) and mineralocorticoids, which control the level of excretion of water and salt by the kidney. If the adrenal cortex does not function adequately, one result is Addison's disease, characterized by hypoglycemia, weakness, and increased susceptibility to stress. The opposite condition, hyperadrenocorticism, is Cushing's syndrome.
33. The hypothalamus secretes hormone-releasing factors. Under the influence of these factors, the pituitary secretes trophic hormones, which act on specific endocrine glands. Individual hormones are then released by the specific endocrine glands.

34. Thyroxine is an amino acid derivative and is absorbed directly from the gut into the bloodstream. If insulin were taken orally, it would be hydrolyzed to amino acids in the stomach and intestine.
35. G proteins get their name because they bind GTP as part of their effect. An example is the G protein that is linked to the epinephrine receptor and leads to the production of cAMP as a second messenger. Receptor tyrosine kinases have a different mode of action. When they bind their hormone, they phosphorylate tyrosine residues on themselves and other target proteins, which then act as a second messenger. Insulin is an example of a hormone that binds to a receptor tyrosine kinase.
36. cAMP, Ca^{2+}, insulin receptor substrate.
37. Human growth hormone is a peptide hormone. If it were taken orally, the peptide would be degraded to its component amino acids in the small intestine and would be rendered useless.

24.4 Hormones and the Control of Metabolism

38. Epinephrine and glucagon are the two that were discussed the most in this book.
39. Glucagon causes the activation of glycogen phosphorylase, inhibition of glycogen synthase, and inhibition of phosphofructokinase-1.
40. Epinephrine has the same affect on glycogen phosphorylase and glycogen synthase, but it has the opposite effect on phosphofructokinase-1.
41. The G protein is bound to GTP. Eventually, the GTP is hydrolyzed to GDP, which causes it to dissociate from adenylate cyclase. This stops the hormone response until the hormone dissociates from the receptor, the G protein trimers are rejoined, and the process starts over again.
42. IP_3 is a polar compound and can dissolve in the aqueous environment of the cytosol; DAG is nonpolar and interacts with the side chains of the membrane phospholipids.
43. When a stimulatory hormone binds to its receptor on a cell surface, it stimulates the action of adenylate cyclase, mediated by the G protein. The cAMP that is produced elicits the desired effect on the cell by stimulating a kinase that phosphorylates a target enzyme.
44. See Table 24.2.
45. It is most unlikely that a metabolic pathway could exist without control mechanisms. Many pathways require energy, so it is advantageous for an organism to shut down a pathway when its products are not needed. Even if a pathway does not require large amounts of energy, the many connections among pathways make it likely that control is established over the levels of important metabolites.
46. In cholera, adenylate cyclase is permanently "turned on." This in turn stimulates active transport of Na^+ and water from epithelial cells, leading to diarrhea.
47. (a) Stoichiometric amounts of cAMP are required to activate cAMP-dependent protein kinase.
    (b) Six catalytic steps, including the reaction catalyzed by glycogen phosphorylase, with 10 molecules acted on in each step, would result in 10^6 (one million) G-1-P molecules for each epinephrine.
    (c) A major factor is speed. It is important to be able to use stored energy rapidly in "fight or flight" situations. A second factor is control. Note that glycogen phosphorylase is activated by kinases. The competing process of glycogen storage, catalyzed by glycogen synthetase, is inactivated by kinases. A third factor is economy. A single molecule of epinephrine activates many molecules of glycogen phosphorylase and yet more molecules of G-1-P.
48. A phosphatase dephosphorylates glycogen phosphorylase and glycogen synthetase, inactivating and activating them, respectively. The phosphatase becomes active in response to high concentrations of glucose.
49. Low-carbohydrate diets are designed to prevent the high blood sugar levels that arise when large quantities of carbohydrates are consumed. High blood sugar leads to a rapid rise in insulin. Insulin is known to stimulate fat synthesis and to inhibit fatty-acid oxidation. Thus, low-carbohydrate diets are thought to help fight weight gain.

24.5 Insulin and Its Effects

50. Insulin's primary function is to stimulate the transport of glucose out of the blood and into the cell.
51. The second messenger is a protein called the insulin receptor substrate, which is phosphorylated on a tyrosine by the insulin receptor kinase.
52. When insulin binds to its receptor, the β-subunit of the receptor kinase autophosphorylates. When this happens, the receptor kinase is able to phosphorylate tyrosines on the insulin receptor kinase.
53. Insulin causes the following effects:
    (a) Glycogen breakdown is decreased.
    (b) Glycogen synthesis is increased.
    (c) Glycolysis is increased.
    (d) Fatty-acid synthesis is increased.
    (e) Fatty-acid storage is increased.
54. Insulin and epinephrine normally have opposite effects, but they both stimulate muscle glycolysis. Epinephrine is the hormone that signals the need for quick energy, which means the muscle cells must be able to use glucose via glycolysis. Insulin stimulates pathways that use up glucose so that the blood glucose lowers, so it makes sense for it to stimulate glycolysis as well. Epinephrine stimulates muscle glycolysis by activating adenylate cyclase, which makes cAMP; cAMP then activates protein kinase A, which phosphorylates phosphofructokinase-2 and fructose-*bis*phosphatase-2. In the muscle, phosphorylation of phosphofructokinase-2 activates it, producing more fructose-2,6-*bis*phosphate, which activates phosphofructokinase-1 and glycolysis. In muscle, insulin stimulates glycolysis by activating phosphofructokinase and pyruvate dehydrogenase.
55. Prerace diet can be critical to a runner. If the race is at 9 AM, and the runner gets up at 7 AM and then eats a typical American breakfast of cereal, toast, or pancakes, she will have a high blood-sugar level within half an hour, which will lead to a high insulin level shortly thereafter. In that scenario, by the time the runner gets to the starting line, she will have a metabolism dedicated to fat and glycogen synthesis and will not be burning fat or carbohydrates. The runner will be like a car with a full tank of gas and a clogged fuel line.
56. It has been shown that the GLUT4 transporter responds to physical activity. When a person is active, the transporter is active and responds well to insulin. After a few days of detraining, this transporter shows only half of the activity it did before.
57. GLUT4 is one of the glucose transporters on muscle cells. It responds to insulin by moving glucose out of the blood and into the cell. In type II diabetes, insulin is present, but it does not have the same effect. It takes more insulin to accomplish the same movement out of the blood and into the cell. People with type II diabetes often show classical signs of obesity, and there is a correlation between diminishing GLUT4 activity, obesity, and diabetes.
58. It had been known for some time that calorie restriction could promote longevity in many species. Scientists discovered a family of proteins called sirtuins that seemed to be at the center of this phenomenon. Sirt1 is a mammalian version of a sirtuin studied very extensively in yeast. This protein is a histone deacetylase, but also seems involved in many other processes. It reacts to scarcity in a way that primes an organism for survival. Transgenic organisms with multiple copies of the gene for Sirt1 show greater longevity and any other stimulus that increases the expression of the gene does too.